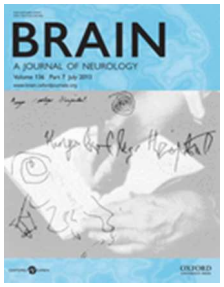

Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans

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Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans

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Abstract

Previously, we reported that one individual who had a motor complete, but sensory incomplete spinal cord injury regained voluntary movement after seven months of epidural stimulation and stand training. We presumed that the residual sensory pathways were critical in this recovery. However, we now report in three more individuals voluntary movement occurred with epidural stimulation immediately after implant even in two who were diagnosed with a motor and sensory complete lesion. We demonstrate that neuromodulating the spinal circuitry with epidural stimulation, enables completely paralyzed individuals to process conceptual, auditory, and visual input to regain relatively fine voluntary control of paralyzed muscles. We show that neuromodulation of the sub-threshold motor state of excitability of the lumbosacral spinal networks was the key to recovery of intentional movement in four out of four individuals diagnosed as having complete paralysis of the legs. We have uncovered a fundamentally new intervention strategy that can dramatically affect recovery of voluntary movement in individuals with complete paralysis even years after injury.

Key words: human spinal cord injury, epidural stimulation, voluntary movement

Abbreviations:

AIS American Spinal Injury Association Impairment Scale

SSEP Somatosensory evoked potentials

TMS Transcranial magnetic stimulation

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Introduction

The clinical diagnosis of having a motor complete lesion commonly classified by the American Spinal Injury Association Impairment Scale (AIS) as grade A or B (Marino et al. 2003; Waring et al. 2010) is when there is no clinical evidence of volitional activation of any muscle below the lesion. Presently, the prognosis of recovery of any intentional control of movement below the injury level after clinically defined complete paralysis for over two years is negligible. This diagnosis has immediate and severe implications for the patientoften limiting the rehabilitation and interventional strategies provide that are targeted for recovery (Burns et al. 2003; Calancie et al. 2004b; Ditunno, Jr. 1999; Waters et al. 1992; Waters et al. 1998). There is not currently an effective treatment that would result in regaining voluntary motor function for these individuals. The presumed solution ultimately has been thought to be to promote regeneration of axons across the lesion with the hope that functionally beneficial connections can be formed (David and Aguayo 1981; Richardson et al. 1980; Zhao et al. 2013).

However, we previously reported the return of control of movement in one individual with motor complete but sensory incomplete spinal cord injury more than two years after complete paralysis following seven months of intense stand training using epidural stimulation (Harkema et al. 2011a). These unexpected results led us to theorize that the residual sensory pathways were critical in mediating the voluntary movements elicited with epidural stimulation and specific intent by the individual. The intense stand training and repetitive stimulation may have driven neural plasticity that eventually resulted in the ability to voluntary move the legs. In this study, we include three additional chronic spinal cord injured individuals, another AIS B (sensory incomplete) and two AIS A (sensory and motor complete) individuals who were also classified as motor complete using all currently available clinical and neurophysiological testing

(Dimitrijevic et al. 1992a; McKay et al. 1997; McKay et al. 2005). We tested their ability to move voluntarily with epidural stimulation after implantation (prior to any training with stimulation) and then again after the intense stand training using epidural stimulation intervention (see supplementary materials for description of stand training) and following intense step training in combination with epidural stimulation. We hypothesized that the AIS A spinal cord injured individuals would not elicit any voluntary movement using epidural stimulation even after training and that the AIS B spinal cord injured individual would develop the ability to voluntarily move the legs only after training. These experiments were conducted as part of a larger ongoing study of stand and step training in combination with epidural stimulation in individuals with chronic motor complete spinal cord injury.

Materials and Methods

Characteristics of Subjects

An epidural spinal cord stimulation unit (Medtronics, RestoreADVANCED) and a 16-electrode array was implanted at vertebrae T11-T12 over spinal cord segments L1-S1(Harkema et al. 2011a) in four individuals with motor complete spinal cord injury (Table 1 and Supplementary Fig. 1) using the following inclusion criteria: 1) stable medical condition without cardiopulmonary disease or dysautonomia that would contraindicate standing or stepping with BWST; 2) no painful musculoskeletal dysfunction, unhealed fracture, contracture, pressure sore, or urinary tract infection thatmight interfere with stand or step training; 3) no clinically significant depression or ongoing drug abuse; 4) no current anti-spasticity medication regimen; 5) non-progressive SCI above T10; 6) AIS A or B; 7) no motor response present in leg muscles during transmagnetic stimulation; 8) not present or bilateral delay of sensory evoked potentials; 10) No volitional control during voluntary movement attempts in leg musclesas measured by

EMG activity; 11) segmentalreflexes remain functional below the lesion; 12) brain influence on spinal reflexes is not observed as measured by EMG activity; 13) must not have received botox injections in the prior six months; 14) be unable to stand or step independently; 15) at least one-year post injury; and 16) must be at least 18 years of age

All four individuals implanted were male, at least two years post date of injury and ranged in neurological level from C7-T5. The average age of all individuals was 26.9 (+/- 4) years at time of implant. All individuals were unable to stand or walk independently or voluntarily move their lower extremities despite standard-of-care rehabilitation and additional intensive locomotor training (Harkema et al. 2011b). The research participants signed an informed consent for electrode implantation, stimulation, stand and stepping training interventions and physiological monitoring studies approved by the University of Louisville and the University of California, Los Angeles Institutional Review Boards.

Table 1: Clinical characteristics of four research participants

Subject	Age (yr)	Gender	Time since injury (yr)	Injury Level	Neuro Level	AIS grade	SSEP		TMS
							Upper	Lower	
B07	23.8	Male	3.4	C7	T2	B	Normal	BD	NR
A45	24	Male	2.2	T5-T6	T4	A	Normal	NP	NR
B13	32.8	Male	4.2	C6-C7	C7	B	Normal	BD	Not tested
A53	27	Male	2.3	T5	T5	A	Normal	NP	NR

SSEP-somatosensory evoked potential; TMS-transcranial magnetic stimulation; C-Cervical; T-Thoracic; BD-Bilateral Delay; NP-Not present; NR-No Response;

Clinical and Physiological Status

Standard of care clinical evaluations were also performed to characterize the injury. Two clinicians independently perform a physical exam and used the American Spinal Injury Association Impairment Scale to classify the injury clinically(Marino et al. 2003; Waring et al. 2010). Two individuals were classified and confirmed as AIS-B and two individuals were classified and confirmed as AIS-A, prior to implantation and at the time of initial lumbosacral spinal cord epidural stimulation (see Supplementary Fig. 1).

Upper and lower extremity somatosensory evoked potentials were assessed clinically prior to implantation and at multiple time points following implant in all participants(Dimitrijevic et al. 1983b; Perot and Vera 1982)(see Supplementary Fig. 1). All participants had normal somatosensory evoked potentials (SSEP) from upper extremity median nerve stimulation at the wrist. Both AIS-B individuals had bilateral cortical delays present from lower extremity stimulation at the posterior tibial nerve and ankle, while both AIS-A individuals had no response (Supplementary Fig. 1). Transcranial magnetic stimulation (TMS) was used clinically to assesses the functional integrity of the cortico-spinal tracts (Dimitrijevic et al. 1992a;McKay et al. 1997;McKay et al. 2005). No motor evoked potentials in the leg muscles were detected during transcranial magnetic stimulation of the motor cortex in tested individuals. A single pulse was delivered through a dual-cone coil placed slightly off center from the scalp vertex to optimize left and right hemispheres. Pulses of 100 microsecond duration were delivered starting at 30% intensity increasing by 5% until 85% of maximum intensity was reached. Data for A45 (post step training with epidural stimulation) and A53 (prior to implantation) are shown in Fig. 1 A and C respectively. Individuals were asked to attempt a sustained dorsiflexion during which TMS was delivered following the same procedures described above. No motor evoked responses in leg muscles were detected during the combined voluntary attempt and TMS and no movement resulted from the attempted action (Fig. 1B and D). B13 was not tested prior to implantation.

Research participants A45 and A53 were classified as motor and sensory complete and B07 and B13 as motor complete and sensory incomplete based on all clinical and neurophysiologic measures described above.

Prior to the electrode implantation, participants received a minimum of 80 locomotor training sessions (Harkema et al. 2012b) using body weight support on a treadmill with manual facilitation. Average body weight support throughout all sessions for all participants was 43.6% (+/- 4.5) walking at an average speed of 1.07 m/s (+/- 0.04). B07 and A45 had no significant EMG activity during stepping. B13 and A53 had significant muscle activity during stepping. There were no significant changes in the EMG activity during stepping following the stepping intervention prior to implantation in any of the four research participants (Harkema et al. 2012a). Some neurophysiological measures can be used to detect residual descending input to the spinal circuitry even when there is no ability to voluntarily execute movement below the level of lesion and these observations have been used to reach a diagnosis of "discomplete" (Calancie et al. 2004a;Dimitrijevic et al. 1984;Dimitrijevic 1994;McKay et al. 2004;Sherwood et al. 1992). A functional neurophysiological assessment was performed at multiple time points prior and following implantation (Fig. 2) to assess residual motor output present during various maneuvers (Dimitrijevic 1988;Dimitrijevic et al. 1992b;Li et al. 2012;McKay et al. 2004;McKay et al. 2011). Supplementary Fig. 2 illustrates motor activity observed during five minute relaxation, a reinforcement maneuver (Dimitrijevic et al. 1983a;Dimitrijevic et al. 1984) and attempts to actively move the ankle joint and hip joint at different time points prior and following implantation. EMG activity during attempts to move the legs or during reinforcement maneuvers was similar to the EMG seen during five minutes of relaxation in all individuals. In B13 and A53 mean EMG amplitudes were higher during attempted relaxation compared to B07 and A45 due to higher levels of resting excitability. In A45 relaxation excitability increased post stand training

with epidural stimulation, however no changes were seen in the ability to activate motor neurons below the level of the injury during active voluntary attempts. No functional connectivity between the supraspinal and spinal centers below the level of injury was detected with clinical or neurophysiological assessments in any of the four subjects.

Assessment of Voluntary Movement

B07 was the first research participant to be implanted and we did not discover his ability to perform voluntary movements of the legs with epidural stimulation until the conclusion of the stand training intervention using epidural stimulation. We then designed and conducted the initial experiments to assess this individual'svoluntary function. Subsequently, the three additional research participants were implanted and prior to beginning any training interventions we conducted the experiments assessing voluntary movement (T1; see Fig. 2). As a result we have different time points for voluntary experiments in relation to training interventions for the first participant compared to the other three participants. Refer to the summary table for a list of experimental sessions and testing time point used on each figure for each research participant (Supplementary Table 1).

We collected EMG, joint angles, and tensile force data at 2,000 Hz using a 24–channel hard-wired AD board and custom-written acquisition software (LabView, National Instruments, Austin, TX). Bilateral EMG (Motion Lab Systems, Baton Rouge, LA) from the soleus, tibialis anterior, medial hamstrings, vastus lateralis, adductor magnus, gluteus maximus, and intercostal (6th rib) muscles was recorded using bipolar surface electrodes with fixed inter-electrode distance (Harkema et al. 1997). Bilateral EMG from the iliopsoas, extensor hallucis longus, and extensor digitorum longus was recorded with fine-wire electrodes. Two surface electrodes

placed symmetrically lateral to the electrode array incision site over the paraspinal muscles were used to record the stimulation artifact. Hip, knee, ankle and first toe joint angles were acquired using a high-speed optical motion capture system (Motion Analysis, Santa Rosa, CA). EMG data were rectified and high-pass filtered at 32 Hz using Labview software customized by our laboratory. Tensile force was measured using a piezoelectric load cell (Kistler, Amherst, NY) mounted on a frame placed around the mat table.

Stimulation parameters were optimized for each leg and joint movement. Cathodes and anodes were selected to target primary motor pool activation areas that were key in the movement. Stimulation was constant and all participants achieved movement at optimal frequencies of either 25 Hz or 30 Hz. Voltage was typically tested through a wide range from sub movement threshold to above optimal. Although initially configuration parameters were specific to a single joint movement and right or left leg, following training, participants were able to perform multiple movements on left and right legs using the same configuration (Supplementary Table 2).

The participants were in a supine position throughout all recordings. To measure force during extension of the first toe a ring was secured over the toe and attached to a force transducer via a non-elastic cable. Similarly the ring and cable were secured around the distal portion of the foot when recording force during ankle dorsiflexion. To record force during flexion of the knee and hip combined the cable was secured to the ankle. The cable had sufficient compliance to allow the leg to move from full extension to a position in which the knee and hip could reach less than a 30° angle. The resistance to the movement was equivalent to the weight of the cable (0.16 N) and the friction force of the foot with the table in the early phase of the leg flexion. A computer monitor displayed a real time sine wave with frequency of 0.25 Hz as well as the force measured by the load cell as the research participants performed the requested action. Both signals were used to determine the degree to which the individual could translate the timing of the visual

appearance of the sine wave to an analogous change in force. Although the sine wave was continuously displayed to the participants, they were instructed to initiate each effort with the rising phase of a sine wave of their choosing. There was no instruction to match the amplitude of force generation with the peak of the sine wave, and there was no verbal command given prior to the attempts.

To assess the ability to translate an auditory signal to an analogous change in force a signal from a tone generator (300 Hz tone frequency), modulated at 0.25 Hz was sent through headphones. Three different volumes (60, 70 and 80 dB) were presented in a random order. The research participant was instructed to modulate the force based on the modulation frequency of the sound and the given amplitude. A trial period was conducted so the participant could listen at the three different volumes and learn to discriminate between them. Full leg flexion was the only action tested with the auditory cues. Stimulation parameters used during these trials were matched to those found as optimal during the visual cue assessment for the same leg.

During experimental sessions continuous stimulation was provided with optimal parameters during all attempts. Stimulation was shut down only to change configurations and to assess the ability to move with no stimulation at the beginning of trials for each joint and side. Experimental sessions lasted for approximately two hours and left/right leg, ankle and toe are tested staring by assessing threshold and optimal stimulation parameters using visual cues. Each joint was also assessed for fine motor control and accuracy including the modulation of three levels of force generation, fast oscillations and sustained force.

Voluntary Movement Training program

A home-based stimulation protocol was developed to allow the individuals to stimulate for approximately 1 hour while practicing intentional movement in the supine position. This protocol started immediately after the first assessment of successful voluntary movement was obtained (Fig. 2). All research participants were asked to practice daily (7 days a week) for one hour. The stimulation protocol for the home-based program was determined in the laboratory. The individuals modulated the voltage needed to optimize each movement after the stimulation configurations were selected in each individual to optimize the voluntary movement of the whole leg, ankle and toe.

Results

Four individuals diagnosed with clinically motor complete paralysis and implanted with a lumbrosacral spinal cord stimulator at least 2.2 years post injury (3.0 +/-0.95 years) were able to execute intentional movements of the legs in response to a verbal command. No motor activity was present when attempting to move without epidural stimulation following a verbal command (B07) or a visual cue (B13, A45 and A53) (Fig. 3A). However, all four individuals were able to generate EMG activity and movement during ankle dorsiflexion in the presence of epidural stimulation (Fig. 3B) during their first experimental session (T1 (prior to stand training): A45, B13, A53 and Post stand training: B07 , see Fig.2).

Appropriate activation and movement of ankle and toe muscles was achieved by all individuals when performing ankle dorsiflexion with epidural stimulation (Fig. 3B). In one subject (B13) clonic-like activity in the toe extensors was present during the movement and sustained clonic activity remained when commanded to relax (Beres-Jones et al. 2003). However, this clonic activity did not prevent the movement. Subjects A45 and A53 had tonic activity in the soleus

(SOL) prior to the attempt, showing a reduction in amplitude during the dorsiflexion effort. Reciprocal inhibition of antagonist muscles was also present in the execution of other movements such as leg flexion. These results demonstrate that humans diagnosed with complete motor paralysis can recover volitional motor drive which can drive coordinated, task-specific movements in the presence of lumbosacral spinal cord epidural stimulation.

Force and rate of modulation.

Having observed some recovery of the ability to move intentionally by each of the four individuals, we began to assess the motor control fidelity. Graded levels of force were generated on command by three out of four individuals (Fig. 4A and B). Three subjects (B07, A45, A53) generated forces that proportionately matched the percent of effort requested in flexing the entire leg (Fig. 4B). The level of activation of the iliopsoas muscle reflected the relative magnitude of force generated during leg flexion. The activation of the intercostal muscles (6th rib), was closely synchronized with the initiation of force generation, generally reflecting the percent effort in stabilizing the rib cage (Fig 4A). Reciprocity in the EMG activity of extensors and flexors when flexing and extending the leg further indicates that the neural activation specificity to the lumbosacral motor pools was sufficient to generate a functionally coordinated movement (Fig. 4A and C). When subject A45 was asked to generate a sustained contraction for 'as long as possible' the effort could be maintained for a few seconds followed by several oscillatory movements (Fig. 4C). There was alternating activity in the iliopsoas and vastus lateralis, with the vastu slateralis only becoming activated after flexor activity ended. Subjects B07 and A53 were also able to perform sustained leg flexion. In all cases, the integrated force was closely linked to the iliopsoas EMG amplitude (Fig. 4D). A53 demonstrated a similar pattern of alternating activity between the iliopsoas and vastus lateralis as shown by the other AIS-A

individual (A45). B13 was unable to generate graded levels of force or a sustained effort prior to training and could not consistently discriminate low and medium efforts following training.

To further assess the level of control of the newly acquired ability to move in the presence of epidural stimulation the individuals were then asked to oscillate as rapidly as possible. The cycle rate for the toe was about 1 Hz for subject B07 (Fig. 5A-C) and approximately 0.4 Hz for subject A45 and 0.85 Hz for A53 when oscillating the whole leg (Fig. 5D-G, Movie S4). Clonic activity (6.5 Hz) of the extensor hallicus longus was not linked to the stimulation frequency (30 Hz) and occurred throughout the test in subject B07 (Fig. 5B).EMG responses were initiated just before there was a rise in force (Fig. 5C). There was no periodic bursting of EMG activity within the timeframe of a single force effort (Fig. 5C). As noted earlier A45 and A53 had reciprocity in the modulation of the EMG of the iliopsoas and the vastus lateralis during leg flexion and extension (Fig. 5D-G). In figure 5D, one of the oscillatory cycles has been marked in red and green which denote the modulation of the amplitude of the EMG of the iliopsoas and vastus lateralis. Figure 5E shows the relative modulation of the hip flexor (IL) and knee extensor (VL), and also highlights the one oscillation with flexion in red and extension in green (A45). Subject A53 showed a similar modulation of the iliopsoas and vastus lateralis during multiple oscillatory cycles of leg flexion (Fig. 5F-G). Following home based training subject B07 was able to generate similar reciprocal activation of flexors and extensors between the iliopsoas and vastus lateralis, but only during single attempts. Subject B13 was not able to generate different levels of force, oscillations or sustain the contractions upon request at his initial testing. His level of clonus and spasticity throughout the day was markedly higher than the other individuals.

Visual and auditory processing.

We also assessed whether volitional control could modulate the level of activation of the appropriate motor pools based on visual and/or auditory input. All individuals could modulate the motor tasks according to visual and auditory cues. The individuals were asked to synchronize the flexion of the leg, dorsiflexion of the ankle and extension of the toe according to the rise and fall of a sine wave displayed on a computer screen. The individuals were able to consistently activate the appropriate muscles for the specified action with temporally synchronized force generation (data not shown). We compared the ability of the four individuals to modulate the flexion of the leg to a visual cue during optimal stimulation to three volume levels (60, 70, 80 dB) of a similar auditory cue (Fig. 6). Individuals were asked to generate leg flexion in response to the onset of a 0.25 Hz auditory signal with the level of force to be generated to correspond to the amplitude of the tone. Data shown in figure 6 are for the following time points, B07 and A45: T3, B13: T2 and A53: T1 (refer to Supplementary Table 1 and Fig.2). Three out of the four individuals were able to discriminate between sound amplitudes, although the differentiation between low and medium forces was not consistent in B13 and B07. For A45 and B07, EMG amplitude of the iliopsoas and the adductor for the high volume was comparable with the EMG amplitudes generated when the force was modulated by a visual cue using the same stimulation parameters. The results demonstrate that auditory and visual cues were processed by the sensorimotor cortex so that the appropriate spinal interneuronal systems below the level of injury enabling the subject to titrate the desired level of excitability of the correct motor pools for the intended movement.

Effects of repetitive training on voluntary performance.

Daily training using epidural stimulation with stand training and home-based voluntary training with epidural stimulation resulted in the generation of voluntary efforts with higher forces and

lowerstimulation voltages to reach the thresholds which enabled voluntary motor responses in two individuals (B07 and B13). After 28 weeks of home-based stand and voluntary training with epidural stimulation in B07, the stimulation threshold for force generation was lower. The threshold intensity was further reduced after an additional 12 weeks of training (Fig. 7A) Similar results were observed in both legs and during toe extension for B07 (data not shown). Following stand training and home-based voluntary training (see Fig.2), B13 showed a similar trend of lower threshold for force generation even achieving movement with no stimulation. With continued home-based voluntary training and at the conclusion of step training, B13 maintained the ability to perform leg flexion with no stimulation. The peak force to stimulation strength relationship for B13 post step training was characteristic of a parabolic function. Stimulation amplitudes between 0.5V and 1.5V resulted in force values lower than those obtained when moving with no stimulation. From 1.5V to 2.5V force increased linearly with increased intensity. In the case of A45, although a force increase was not observed at lower stimulation voltages following training, improvements in the accuracy to match the oscilloscope signal were observed (Fig. 7B).

Figure 7B compares the ability of all subjects to match force generation during leg flexion to a visual cue at T1 (top panel), T2 (middle panel) and T3 (bottom panel). A45 showed the greatest accuracy improvement matching the force generation to the visual cue (Fig. 7B). This accuracy was demonstrated both while responding to a visual cue, as well as to an auditory cue. A53 is currently undergoing steptraining, therefore T3 data has not been collected. These results demonstrate the ability of the spinal networks to learn with task specific training and improve motor pool recruitment to promote force generation and accuracy.Another representation of the volitional contributions in a more task specific activity was demonstrated by A45 and A53 while stepping on the treadmill with body weight support and manual assistance, in the presence of

epidural stimulation (Fig. 8). During stepping A45 was able to modulate the amount and pattern of EMG activity of lower extremity muscles when consciously thinking about stepping and moving the legs through the step cycle (Fig. 8A). Amplitude and burst duration of the flexors and extensors (bilaterally) were increased, extensors of the left knee and bilateral ankles were also modulated by the intent. Linear envelop plots of flexors and extensors show a general increase in amplitude although the coordination was not changed (Fig. 8B). Similarly, A53 was able to modulate the amplitude of EMG of flexors and extensors during the step cycle (Fig. 8C). Both participants were able to have a greater modulation of the flexor groups as compared to the extensors. This is consistent with their voluntary activity practice as all participants regained the ability to perform flexion tasks with a greater difficulty performing active extension. This ability to modulate EMG with intent during stepping was only seen in two out of three individuals. Due to the fact that this observation was performed following the completion of all laboratory training for B07, we never tested his ability to modulate motor output during stepping. B13 was not able to show this ability during stepping,this might be result of the higher sensitivity to stimulation and greater difficulty in performing multiple repetitions of volitional activity.

Discussion

This study demonstrates the ability of four individuals with chronic complete motor paralysis to execute voluntary tasks with selectivity of appropriate motor poolsin the presence of epidural stimulation. High fidelity sensorimotor translation of visual and auditory signals were processed to control the timing and amount of force generated during the movements. In three out of the four individuals, we observed the recovery of voluntary movement with epidural

stimulation soon after implantation, two of whom had complete loss of both motor and sensory function (Movies S1-3). This shows that by neuromodulating the spinal circuitry at sub-threshold motor levels with epidural stimulation, chronically complete paralyzed individuals can process conceptual, auditory, and visual input to regain specific voluntary control of paralyzed muscles. We have uncovered a fundamentally new intervention strategy that can dramatically affect recovery of voluntary movement in individuals with complete paralysis even years after injury.

The results in the three individuals who were tested after implantation but before repetitive training suggests that descending connections to the spinal cord circuitry may have existed since the time of injury. Subject B07 did not show voluntary ability until after 7 months of epidural stimulation and stand training, from an attempt he initiated. The experimental protocol did not include attempted voluntary movements at that time so we cannot verify whether movement was possible before that time. However, subjects A45, B13, A53 were able to voluntarily execute movements after 11, 4 and 7 days of epidural stimulation, respectively (see Supplementary Table 1). Anatomical connections may have persisted following the injury that were "silent" previously because of loss of conduction due to disruption of myelin or the ionic channels of the neurons (Coggan et al. 2011;Fehlings and Nashmi 1996;Shi and Blight 1997;Sinha et al. 2006;Waxman 1989). In our study, these individuals initially were able to elicit these intentional movements only with the epidural stimulation indicating that the available supraspinal connections limited influence was not sufficient to activate motor pools. As demonstrated in Fig. 1B and D, TMS did not show any changes in excitation even when the individuals were requested to attempt to actively dorsiflex the ankle at the time the TMS pulse was delivered.This suggests that the alteration of the spinal cord circuitry with epidural stimulation was enhancing the central excitatory drive to the motorneurons and conceivably via activated lumbosacral interneurons since prior to the intent of the movement the motor pools

were not active (Angel et al. 1996;Angel et al. 2005;Bannatyne et al. 2009;Cabaj et al. 2006;Djouhri and Jankowska 1998;Edgley et al. 2004). The SSEP results in the two AIS-B individuals showing a shorter latency of responses might also suggest improved connectivity promoted by repetitive epidural stimulation and improved somatosensory transmission.

The resting general excitability state of the spinal circuitry was different among the four individuals that participated in this study (Supplementary Fig.2). There were differences in stimulation intensity thresholds seen across individuals for the generation of movement (Fig.7). B07 and A45 required higher levels of stimulation to initiate movement and had relatively low levels of clonus and spasticity throughout the day. While B13 and A53, the more spontaneously active individuals who reported more clonus and spasticity, could initiate movement at lower thresholds of stimulation. We propose that the functional state of spinal network excitability of interneurons(Bannatyne et al. 2009) and motor neurons was modulated by the epidural stimulation, presumably driving them closer to their appropriate activation threshold, enabling intentional movement (Berg et al. 2007;Quevedo et al. 2005;Yarom and Hounsgaard 2011). Thus, loss of voluntary control of movement may be attributed to not only a physical disruption of descending connections, but also to a physiological alteration of the central state of excitability of the spinal circuitry (de Leon et al. 1999;Edgerton et al. 1997;Tillakaratne et al. 2002). These findings have important implications regarding the significance of sub-motor threshold modulation of spinal circuitry as an important factor in motor control in the uninjured as well as injured spinal cord.

The recovery of the volitional motor drive in four individuals diagnosed with complete motor paralysis (Fig.3) could have been facilitated by lumbosacral spinal cord epidural stimulation via transmission of rostrocaudal signal propagation through the propriospinal interneuronal projections(Courtine et al. 2008;Cowley et al. 2008;Flynn et al. 2011;Zaporozhets

et al. 2006;Zaporozhets et al. 2011). We assessed the subjects' ability to execute a movement on command by comparing the EMG and force patterns with a sine wave pattern on a computer screen (Fig.7B). In general, at the first time point tested, all individuals demonstrated a delay in the initiation of the force relative to the rise of the sine wave and the force decline was generally significantly earlier than the decline of the position cursor. Regardless of these time differences in initiation and termination of the effort the subjects could clearly execute the movements on command. The delay in the motor response related to the intention to move as was observed in each individual couldbe consistent with the voluntarily controlled motor commands being mediated more indirectly through descending propriospinal pathways. Although we cannot rule out a delay in processing the auditory and visual cues.There is precedence in the rat model that tonic intraspinal stimulation immediately caudal to the injury resulted in functional improvements comparable with those seen following long-distance axon regeneration (Yakovenko et al. 2007). Cowley and colleagues showed that even in the absence of long direct transmission, propriospinal pathways through commissural projections can transmit a locomotor command to the lumbrosacral spinal cord with 27% success (Cowley et al. 2010). Thus, the stimulation may facilitate excitation of propriospinal neurons which supports propagation of the voluntary command to the lumbosacral spinal cord.

It is also possible that reticulospinal neurons may mediate ipsilateral corticospinal tract relay via commissural interneurons. Studies have shown that commissural interneurons activated by reticulospinal neurons have direct contacts withinterneurons mediating reflex actions from group Ib tendon organ afferents and group II muscle spindle afferents and modulate actions of these interneurons. (Cabaj et al. 2006;Edgley et al. 2004).The strengthening of reticulospinal connections to motor neurons was demonstrated in monkeys following an unilateral corticospinal tract lesion (Zaaimi et al. 2012). Future studies are needed to probe

pathways including propriospinal, corticospinal, reticulospinal and vestibulospinal that have residual connectivity and can support the propagation of signals.

In all four research participants, their ability to voluntarily move improved over time with daily epidural stimulation and voluntary training while also receiving stand or step training. Figure 7 shows the effect of training on the ability to generate higher forces with similar stimulation intensities as well as more accurately match a visual cue. These results demonstrate the ability of the spinal networks to learn with task specific training and improve motor pool recruitment to promote force generation and accuracy. Conceivably, after repetitive epidural stimulation and training, plasticity of these disrupted pathways could have resulted in a more functional state. The newly established functional connectivity presumably involves multiple, novel neuronal pathways and synapses (Raineteau et al. 2002).Exercise training after spinal cord injury in a rodent model has been shown to enhance corticospinal tract sprouting and increased functional connections to spinal neurons (Bareyre et al. 2004;Courtine et al. 2008;Engesser-Cesar et al. 2007;Flynn et al. 2013;Goldshmit et al. 2008). Similarly it is conceivable that sprouting or growth of axons across the lesion occurred in response to repetitive epidural stimulation and/or stand training. Axonal sprouting is unlikely as the mechanism for early execution of the voluntary movement in three out of four individuals studies here.

This study demonstrates that individuals diagnosed as clinically motor complete can develop functional connectivity across the lesion in the presence of epidural stimulation. This emphasizes the importance of resolving the uncertainty associated with classifying a patient as clinically complete and then determining that no recovery is possible (Burns et al. 2003;Calancie et al. 2004a;Ditunno, Jr. 1999;Kakulas 2004;Waters et al. 1998). Although the exact mechanisms which enabled these surprising results cannot be definitively identified, the possibility of re-establishing functionally meaningful voluntary control in the presence of

epidural stimulation places a high priority on reconsideration of the mechanisms contributing to paralysis in humans. These results indicate that epidural stimulation with activity-dependent plasticity can be used to develop effective therapeutic interventions for recovery of movement in individuals with chronic complete motor paralysis.

The acquired ability to have fine motor control and fidelity of movement in the presence of epidural stimulation currently has limitations when attempting to translate functional benefit to daily life. The lack of a usable interactive patient interface with imbedded control algorithms makes the movements as used prior to injury limited. However, all four of these individuals have found unique ways to incorporate their ability to move their trunk and legs into daily activities. For example, they use their trunk and leg epidural stimulation configurations during physical workouts and facets of their daily life allowing them to be more stable, pursue activities for longer periods of time and they report greater strength and less fatigue. We are following these reported activities to guide the design of the control algorithms and patient interfaces. Future experiments with improved technology are needed expediently in order to take the most advantage functionally of these neurophysiological findings in people after severe spinal cord injury.

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Fig.1: Transmagnetic stimulation (TMS) motor evoked potentials of the soleus (SOL) and tibialis anterior (TA) for two subjects (A45 and A53) performed during multiple stimulation intensities without active dorsiflexion (A and C) and during active dorsiflexion (B and D). No responses were seen for either subject at the recorded intensities.

Fig.2: **Timeline of implantation and experimental sessions for all participants.** All individuals underwent a screening phase with at least 80 session of locomotor training prior to implantation. B07 was the first subject implanted and voluntary activity was not found until the end of stand with epidural stimulation (ES). He was tested with EMG at this time point, however T1 represents the first experimental session with force and fine motor control testing. A45, B13 and A53 where tested with the same protocol post implantation and prior to the beginning of stand training with ES. All participants initiated a home training program for voluntary activity after the initial finding of their ability to move with ES. Clinical evaluations including TMS, FNPA, SSEPs and ASIA exams were performed before and after the 80 sessions of locomotor training during the screening phase and at the conclusion of stand and step training with epidural stimulation. Blue arrows show time points where clinical evaluations took place. A53 is currently undergoing step training with ES. Refer to supplementary table 1 and 2 for further detail.

Fig. 3. Lower extremity EMG activity during voluntary movement occurred only with epidural stimulation in four individuals with motor complete spinal cord injury. EMG activity during attempts of ankle dorsiflexion (**A**) without stimulation and (**B**) with stimulation. Force was not collected for B07. Electrode representation for each subject denotes the stimulation configuration used. Although stimulation was applied throughout the time shown in b, in all four subjects EMG bursts were synchronized with the intent to move. Gray boxes are cathodes and black boxes are anodes, white boxes are inactive electrodes. Stimulation frequency varied from 25 to 30Hz. Muscles, surface EMG:Intercostal 6th rib (IC), tibialis anterior (TA), soleus (SOL); fine wire EMG: iliopsoas (IL), extensor digitorum longus (EDL), extensor hallucis longus (EHL). Black bars represent flexion as determined by peak force generation and white bars represent passive extension (from peak force to return to resting position). Although the subjects were instructed to flex and extend, they were unable to actively extend prior to training. Gray highlighted indicates the active 'flexion / extension' period.

Fig. 4. Force level and endurance of voluntary movements. **(A)** Left leg force and left iliopsoas, vastus lateralis and intercostals EMG activity generated during a low (20%), medium (60%) and high (100%) effort of hip/knee flexion with epidural stimulation from A45. Gray shading indicates force duration. (**B**) Integrated leg force (left y-axis) and iliospsoas EMG activity(right y-axis)from subjects A45 (red); B07 (black) and A53 (blue) during hip/knee flexion with epidural stimulation. Solid symbols (with solid line) represent the integrated force for each attempt and open symbols (with dash

line)represent the integrated EMG of the iliopsoas muscle for each attempt. (**C**) Left leg force and iliopsoas, vastus lateralis and intercostals EMG activity generated during a request to sustain voluntary flexion as long as possible from A45. Shaded gray indicates duration of sustained force. (**D**) Integrated force (solid bar) and iliopsoas EMG (open bar) during sustained contraction from subjects A45, B07 and A53 as shown in c. Electrode representation denotes the stimulation configuration used by A45. Gray boxes are cathodes and black boxes are anodes, white boxes are inactive electrodes. Stimulation frequency was 25 Hz. Muscles, surface EMG: Intercostal 6th rib (IC), vastus lateralis (VL); fine wire EMG: iliopsoas (IL). Stimulation artifact recorded over paraspinal muscles at T12 (ES - blue trace). FRC: force measured through load cell and non-elastic wire.

Fig. 5.Rate of voluntary movements controlled by three individuals with motor complete spinal cord injury.(**A**) Force and extensor hallucis longus (EHL) EMG activity during fast voluntary first toe flexion/extension against a compliant resistance from B07 from a single attempt. (**B**) Represents a 1-sec sweep from (A) prior to initiation of force generation encompassed by the first dashed vertical box. Ten 10msec traces of EHL are overlaid every 0.1 sec (bottom panel). The red crosses represent the timing of the stimulation artifact. (**C**) Represents a 1-sec period (A) during one cycle of force generation encompassed by the second dashed vertical box. Traces of EHL are shown as an overlay of 31 responses marked relative to the stimulation stimulus (bottom panel). (**D and F**) Force (black line) and iliopsoas and vastus lateralis EMG activity during fast voluntary whole leg flexion/extension against a compliant resistance from A45 and A53 respectively. The linear envelope of the EMG signals (purple line, filter: second-order Butterworth 500-100Hz) is shown over the raw signal. (**E and G**) Plot of linear envelope

of the vastus lateralis vs. iliopsoas from (D and F). Red over the linear envelopes represents the flexion phase while green represents the extension phase of one cycle of the movement. Electrode representation for each subject denotes the stimulation configuration used. Gray boxes are cathodes and black boxes are anodes, white boxes are inactive electrodes. Stimulation frequency varied from 25 to 30Hz. Muscles, surface EMG: vastus lateralis (VL); *fine wire EMG: iliopsoas (IL), extensor hallucis longus (EHL). Stimulation artifact recorded over paraspinal muscles at T12 (ES - blue trace).

Fig. 6. Voluntary movement with epidural stimulation performed in response to visual and auditory cues in four individuals with motor complete spinal cord injury. Averaged linear envelopes (filter: Winter Butterworth low-pass 2 Hz) of EMG activity and force (FORCE) generated from three trials. The left panel of each participant represents whole leg flexion/extension in response to a visual cue during optimal stimulation. Black line represents the mean signal and gray line indicates one standard deviation about the mean. The red line represents the oscilloscope signal which served as the visual cue. Right panel of each participant represents whole leg flexion in response three different volumes of an auditory cue during optimal stimulation. Black line represents the mean signal and gray line indicates one standard deviation about the mean. The red line represents the oscilloscope signal which matched the auditory volume cue. Stimulation parameters and voltages for the visual and auditory attempts were the same for each subject. Electrode representation for each subject denotes the stimulation configuration used. Gray boxes are cathodes and black boxes are anodes, white boxes are inactive electrodes. Stimulation frequency varied from 25 to 30Hz.Muscles, surface EMG:Intercostal 6th rib (IC), adductor magnus (AD); fine wire EMG: iliopsoas (IL).

Fig. 7.Stimulation voltage to force relationship during voluntary movement with epidural stimulation in three individuals with motor complete spinal cord injury. (A) Relationship between stimulation strength (Voltage, V) and peak force (Newtons, N) during hip/knee flexion for three time points in subject B07, A45, B13 and A53 (only two time points tested). Cubic line of best fit is shown. Electrode representation for each subject denotes the stimulation configuration used. Gray boxes are cathodes and black boxes are anodes, white boxes are inactive electrodes. Stimulation frequency varied from 25 to 30Hz. (**B**) TOP PANEL: Overlap of linear envelopes for IL (filter: Winter Butterworth low-pass 2 Hz) (Green), Force generation (red) and oscilloscope signal (dark blue) for first time point (T1-red) performed at optimal voltage. MIDDLE PANEL: Overlap of linear envelopes (filter: Winter Butterworth low-pass 2 Hz) (Green), Force generation (black) and oscilloscope signal (dark blue) for post stand training (T2-blue) performed at optimal voltage. BOTTOM PANEL: Overlap of linear envelopes (filter: Winter Butterworth low-pass 2 Hz) (Green), Force generation (black) and oscilloscope signal (dark blue) for last time point (T3-black) performed at optimal voltage. Attempts show improvements in accuracy of force generation during hip/knee flexion following a visual cue.

Fig. 8.Modulation in EMG activity during volitional assistance during stepping with epidural stimulation by an individual with motor complete spinal cord. Coordination and amplitude of EMG was modified by the intent to step. **(A)** Left and right activity during continuous stepping (40% BWS, 1.07 m/s) with epidural stimulation and manual assistance. Initial steps show EMG pattern while the subject (A45) is not thinking about stepping. Section within the red dashed lines show the period of steps while the subject is consciously thinking about stepping and facilitating each step (with voluntary intent).

(B) Plot of linear envelope of the EMG signals of the soleus vs. tibialis anterior (top) and rectus femoris vs. medial hamstrings (bottom) (filter: second-order Butterworth 500-100Hz). Black plots represent both the steps before and after the voluntary intent to assist, while red plots represent all steps within the dashed lines during voluntary intent to assistfrom (A). **(C)** Similar data from A53during continuous stepping (40% BWS, 0.36 m/s) with epidural stimulation and manual assistance. Plot of linear envelope of the EMG signals of the soleus vs. tibialis anterior (top) and rectus femoris vs. medial hamstrings (bottom) (filter: second-order Butterworth 500-100Hz). Black plots represent both the steps before and after the voluntary intent to assist, while red plots represent steps in which A53 is consciously thinking about stepping and facilitating each step. Electrode representation denotes the stimulation configuration used. Gray boxes are cathodes and black boxes are anodes, white boxes are inactive electrodes. Stimulation frequency was 40Hz for A45 and 30Hz for A53. Note A53 was using 3 interleaved configurations.Muscles: soleus (SOL), medial gastrocnemius (MG), tibialis anterior (TA), medial hamstrings (MH), vastus lateralis (VL), and rectus femoris (RF). EMG data is presented in mV. Hip, knee and ankle are sagittal joint angles (degrees). The break in kinematic data is the result of a brief interruption in the recording. Ground reaction forces (FSCAN) reflect stance and swing phase (Newtons).

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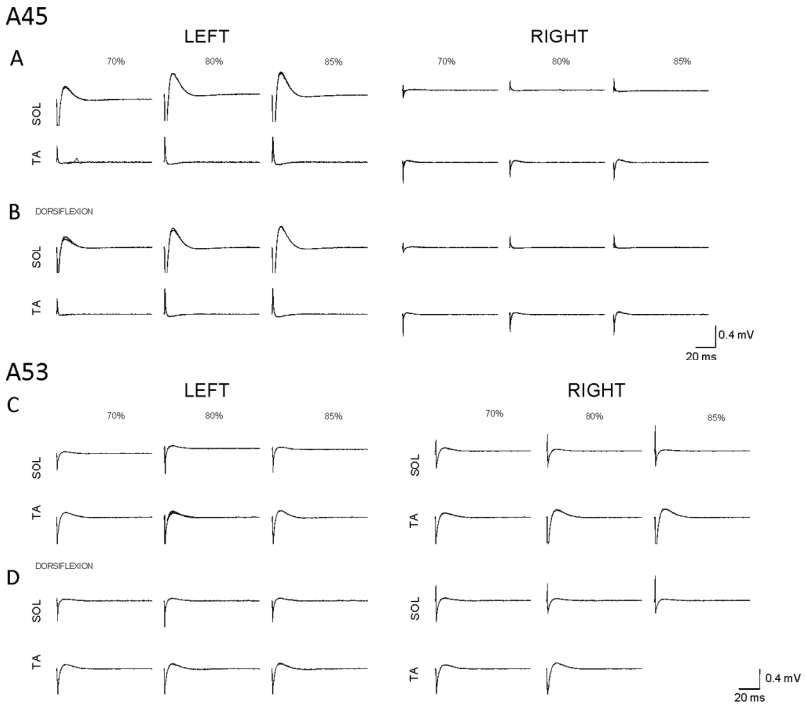

Fig. 1

Fig. 1: Transmagnetic stimulation (TMS) motor evoked potentials of the soleus (SOL) and tibialis anterior (TA) for two subjects (A45 and A53) performed during multiple stimulation intensities without active dorsiflexion (A and C) and during active dorsiflexion (B and D). No responses were seen for either subject at the recorded intensities.
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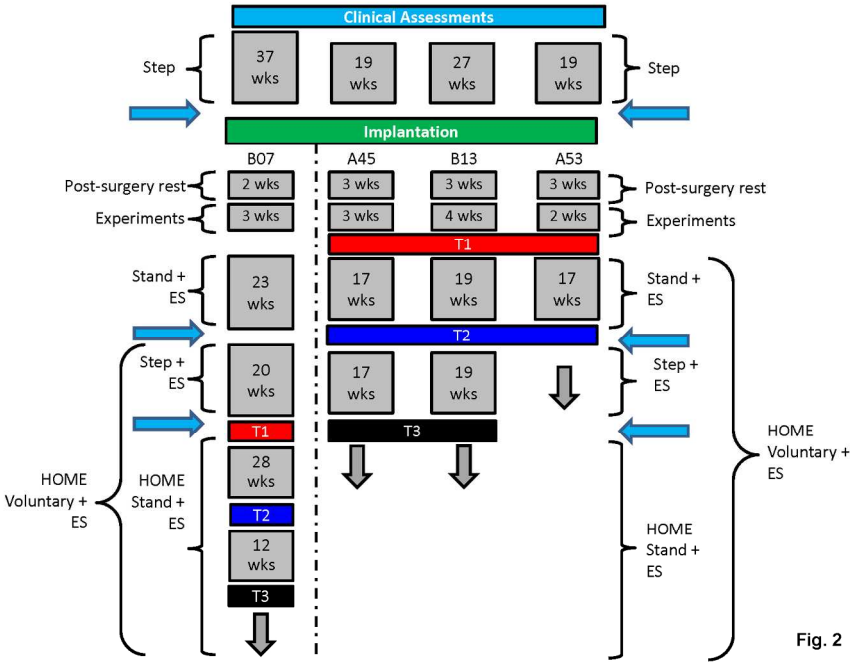

Fig. 2: Timeline of implantation and experimental sessions for all participants. All individuals underwent a screening phase with at least 80 session of locomotor training prior to implantation. B07 was the first subject implanted and voluntary activity was not found until the end of stand with epidural stimulation (ES). He was tested with EMG at this time point, however T1 represents the first experimental session with force and fine motor control testing. A45, B13 and A53 where tested with the same protocol post implantation and prior to the beginning of stand training with ES. All participants initiated a home training program for voluntary activity after the initial finding of their ability to move with ES. Clinical evaluations including TMS, FNPA, SSEPs and ASIA exams were performed before and after the 80 sessions of locomotor training during the screening phase and at the conclusion of stand and step training with epidural stimulation. Blue arrows show time points where clinical evaluations took place. A53 is currently undergoing step training with ES. Refer to supplementary table 1 and 2 for further detail.
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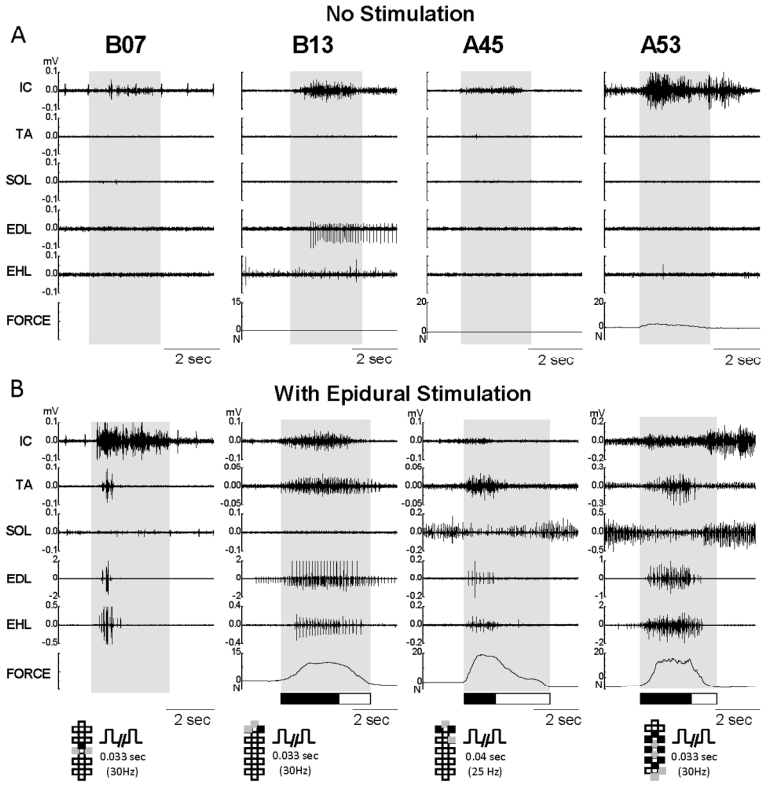

Fig. 3

Fig. 3. Lower extremity EMG activity during voluntary movement occurred only with epidural stimulation in four individuals with motor complete spinal cord injury. EMG activity during attempts of ankle dorsiflexion (A) without stimulation and (B) with stimulation. Force was not collected for B07. Electrode representation for each subject denotes the stimulation configuration used. Although stimulation was applied throughout the time shown in b, in all four subjects EMG bursts were synchronized with the intent to move. Gray boxes are cathodes and black boxes are anodes, white boxes are inactive electrodes. Stimulation frequency varied from 25 to 30Hz. Muscles, surface EMG: Intercostal 6th rib (IC), tibialis anterior (TA), soleus (SOL); fine wire EMG: iliopsoas (IL), extensor digitorum longus (EDL), extensor hallucis longus (EHL). Black bars represent flexion as determined by peak force generation and white bars represent passive extension (from peak force to return to resting position). Although the subjects were instructed to flex and extend, they were unable to actively extend prior to training. Gray highlighted indicates the active 'flexion / extension' period.
215x279mm (200 x 200 DPI)

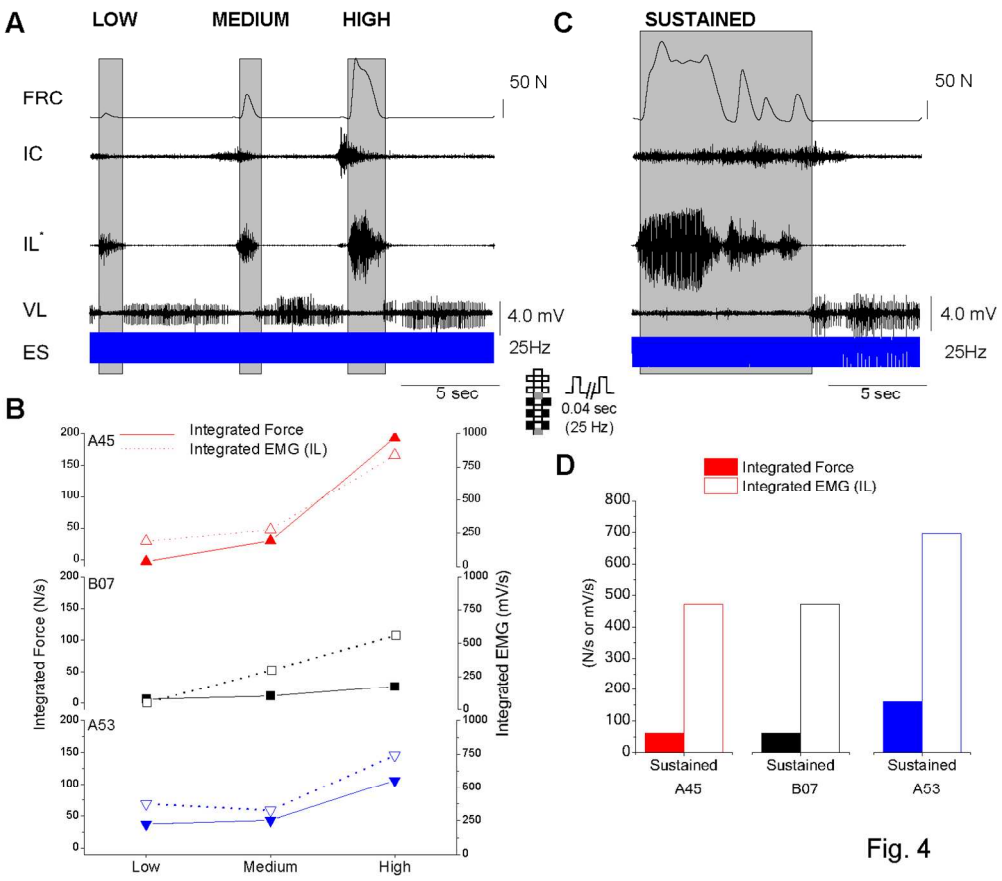

Fig. 4

Fig. 4. Force level and endurance of voluntary movements. (A) Left leg force and left iliopsoas, vastus lateralis and intercostals EMG activity generated during a low (20%), medium (60%) and high (100%) effort of hip/knee flexion with epidural stimulation from A45. Gray shading indicates force duration. (B) Integrated leg force (left y-axis) and iliospsoas EMG activity(right y-axis)from subjects A45 (red); B07 (black) and A53 (blue) during hip/knee flexion with epidural stimulation. Solid symbols (with solid line) represent the integrated force for each attempt and open symbols (with dash line)represent the integrated EMG of the iliopsoas muscle for each attempt. (C) Left leg force and iliopsoas, vastus lateralis and intercostals EMG activity generated during a request to sustain voluntary flexion as long as possible from A45. Shaded gray indicates duration of sustained force. (D) Integrated force (solid bar) and iliopsoas EMG (open bar) during sustained contraction from subjects A45, B07 and A53 as shown in c. Electrode representation denotes the stimulation configuration used by A45. Gray boxes are cathodes and black boxes are anodes, white boxes are inactive electrodes. Stimulation frequency was 25 Hz. Muscles, surface EMG: Intercostal 6th rib (IC), vastus lateralis (VL); fine wire EMG: iliopsoas (IL). Stimulation artifact recorded over paraspinal muscles at T12 (ES - blue trace). FRC: force measured through load cell and non-elastic wire.
199x177mm (200 x 200 DPI)

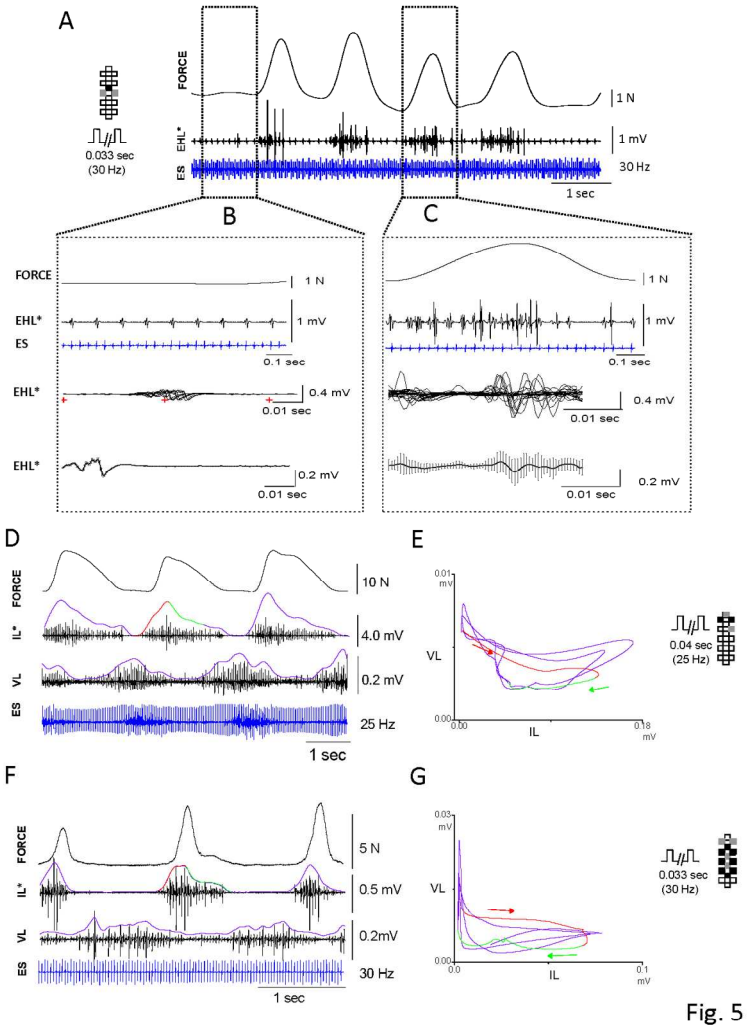

Fig. 5

Fig. 5. Rate of voluntary movements controlled by three individuals with motor complete spinal cord injury. (A) Force and extensor hallucis longus (EHL) EMG activity during fast voluntary first toe flexion/extension against a compliant resistance from B07. (B) Represents a 1-sec sweep from (A) prior to initiation of force generation encompassed by the first dashed vertical box. Ten 10msec traces of EHL are overlaid every 0.1 sec (bottom panel). The red crosses represent the timing of the stimulation artifact. (C) Represents a 1-sec period (A) during one cycle of force generation encompassed by the second dashed vertical box. Traces of EHL are shown as an overlay of 31 responses marked relative to the stimulation stimulus (bottom panel). (D and F) Force (black line) and iliopsoas and vastus lateralis EMG activity during fast voluntary whole leg flexion/extension against a compliant resistance from A45 and A53 respectively. The linear envelope of the EMG signals (purple line, filter: second-order Butterworth 500-100Hz) is shown over the raw signal. (E and G) Plot of linear envelope of the vastus lateralis vs. iliopsoas from (D and F). Red over the linear envelopes represents the flexion phase while green represents the extension phase of one cycle of the movement. Electrode representation for each subject denotes the stimulation configuration

used. Gray boxes are cathodes and black boxes are anodes, white boxes are inactive electrodes. Stimulation frequency varied from 25 to 30Hz. Muscles, surface EMG: vastus lateralis (VL); *fine wire EMG: iliopsoas (IL), extensor hallucis longus (EHL). Stimulation artifact recorded over paraspinal muscles at T12 (ES - blue trace).
215x279mm (200 x 200 DPI)

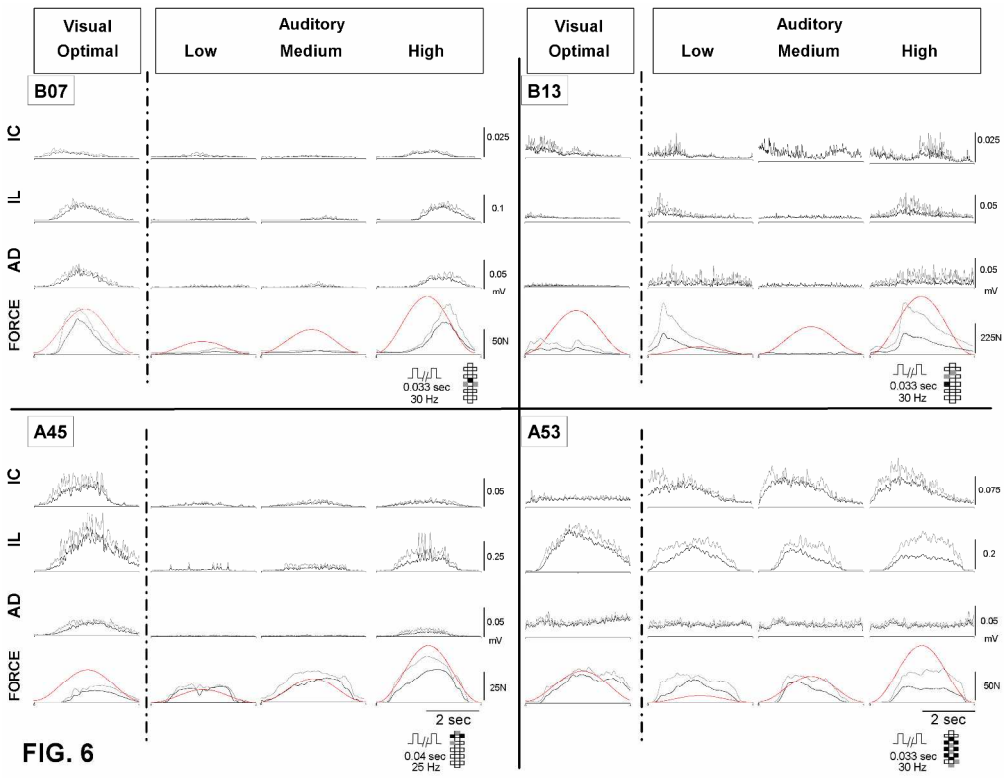

Fig. 6. Voluntary movement with epidural stimulation performed in response to visual and auditory cues in four individuals with motor complete spinal cord injury. Averaged linear envelopes (filter: Winter Butterworth low-pass 2 Hz) of EMG activity and force (FORCE) generated from three trials. The left panel of each participant represents whole leg flexion/extension in response to a visual cue during optimal stimulation. Black line represents the mean signal and gray line indicates one standard deviation about the mean. The red line represents the oscilloscope signal which served as the visual cue. Right panel of each participant represents whole leg flexion in response three different volumes of an auditory cue during optimal stimulation. Black line represents the mean signal and gray line indicates one standard deviation about the mean. The red line represents the oscilloscope signal which matched the auditory volume cue. Stimulation parameters and voltages for the visual and auditory attempts were the same for each subject. Electrode representation for each subject denotes the stimulation configuration used. Gray boxes are cathodes and black boxes are anodes, white boxes are inactive electrodes. Stimulation frequency varied from 25 to 30Hz.Muscles, surface EMG: Intercostal 6th rib (IC), adductor magnus (AD); fine wire EMG: iliopsoas (IL).
267x207mm (300 x 300 DPI)

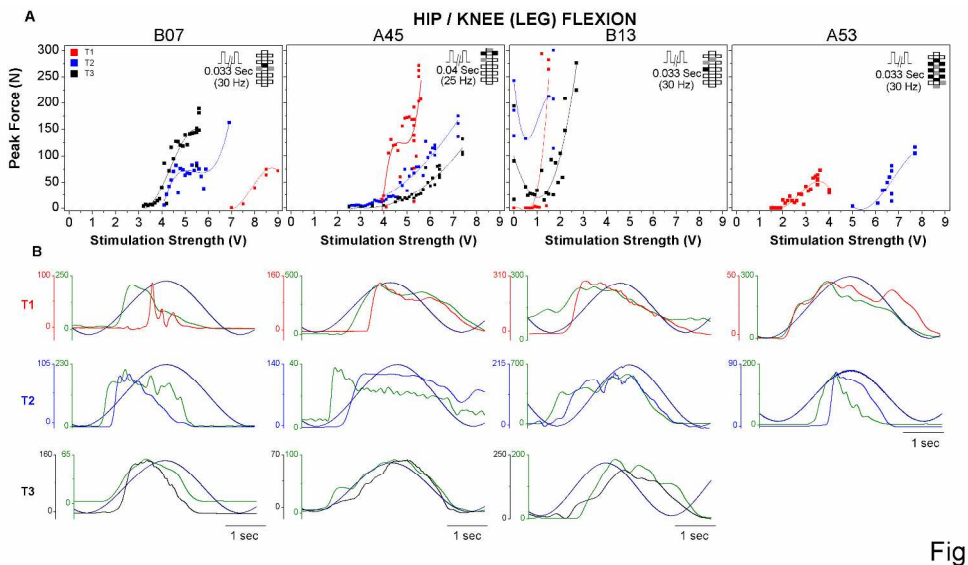

Fig. 7

Fig. 7. Stimulation voltage to force relationship during voluntary movement with epidural stimulation in three individuals with motor complete spinal cord injury. (A) Relationship between stimulation strength (Voltage, V) and peak force (Newtons, N) during hip/knee flexion for three time points in subject B07, A45, B13 and A53 (only two time points tested). Cubic line of best fit is shown. Electrode representation for each subject denotes the stimulation configuration used. Gray boxes are cathodes and black boxes are anodes, white boxes are inactive electrodes. Stimulation frequency varied from 25 to 30Hz. (B) TOP PANEL: Overlap of linear envelopes for IL (filter: Winter Butterworth low-pass 2 Hz) (Green), Force generation (red) and oscilloscope signal (dark blue) for first time point (T1-red) performed at optimal voltage. MIDDLE PANEL: Overlap of linear envelopes (filter: Winter Butterworth low-pass 2 Hz) (Green), Force generation (black) and oscilloscope signal (dark blue) for post stand training (T2-blue) performed at optimal voltage. BOTTOM PANEL: Overlap of linear envelopes (filter: Winter Butterworth low-pass 2 Hz) (Green), Force generation (black) and oscilloscope signal (dark blue) for last time point (T3-black) performed at optimal voltage. Attempts show improvements in accuracy of force generation during hip/knee flexion following a visual cue.
268x151mm (300 x 300 DPI)

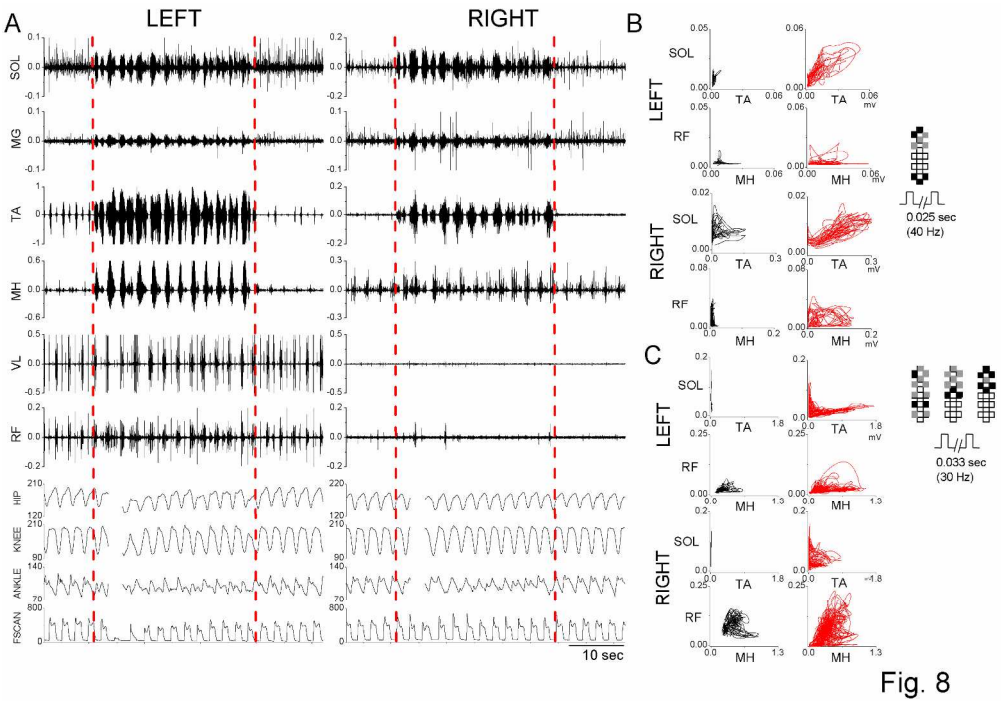

Fig. 8

Fig. 8. Modulation in EMG activity during volitional assistance during stepping with epidural stimulation by an individual with motor complete spinal cord. Coordination and amplitude of EMG was modified by the intent to step. (A) Left and right activity during continuous stepping (40% BWS, 1.07 m/s) with epidural stimulation and manual assistance. Initial steps show EMG pattern while the subject (A45) is not thinking about stepping. Section within the red dashed lines show the period of steps while the subject is consciously thinking about stepping and facilitating each step (with voluntary intent). (B) Plot of linear envelope of the EMG signals of the soleus vs. tibialis anterior (top) and rectus femoris vs. medial hamstrings (bottom) (filter: second-order Butterworth 500-100Hz). Black plots represent both the steps before and after the voluntary intent to assist, while red plots represent all steps within the dashed lines during voluntary intent to assist from (A). (C) Similar data from A53 during continuous stepping (40% BWS, 0.36 m/s) with epidural stimulation and manual assistance. Plot of linear envelope of the EMG signals of the soleus vs. tibialis anterior (top) and rectus femoris vs. medial hamstrings (bottom) (filter: second-order Butterworth 500-100Hz). Black plots represent both the steps before and after the voluntary intent to assist, while red plots represent steps in which A53 is consciously thinking about stepping and facilitating each step. Electrode representation denotes the stimulation configuration used. Gray boxes are cathodes and black boxes are anodes, white boxes are inactive electrodes. Stimulation frequency was 40Hz for A45 and 30Hz for A53. Note A53 was using 3 interleaved configurations. Muscles: soleus (SOL), medial gastrocnemius (MG), tibialis anterior (TA), medial hamstrings (MH), vastus lateralis (VL), and rectus femoris (RF). EMG data is presented in mV. Hip, knee and ankle are sagittal joint angles (degrees). The break in kinematic data is the result of a brief interruption in the recording. Ground reaction forces (FSCAN) reflect stance and swing phase (Newtons).
252x176mm (300 x 300 DPI)

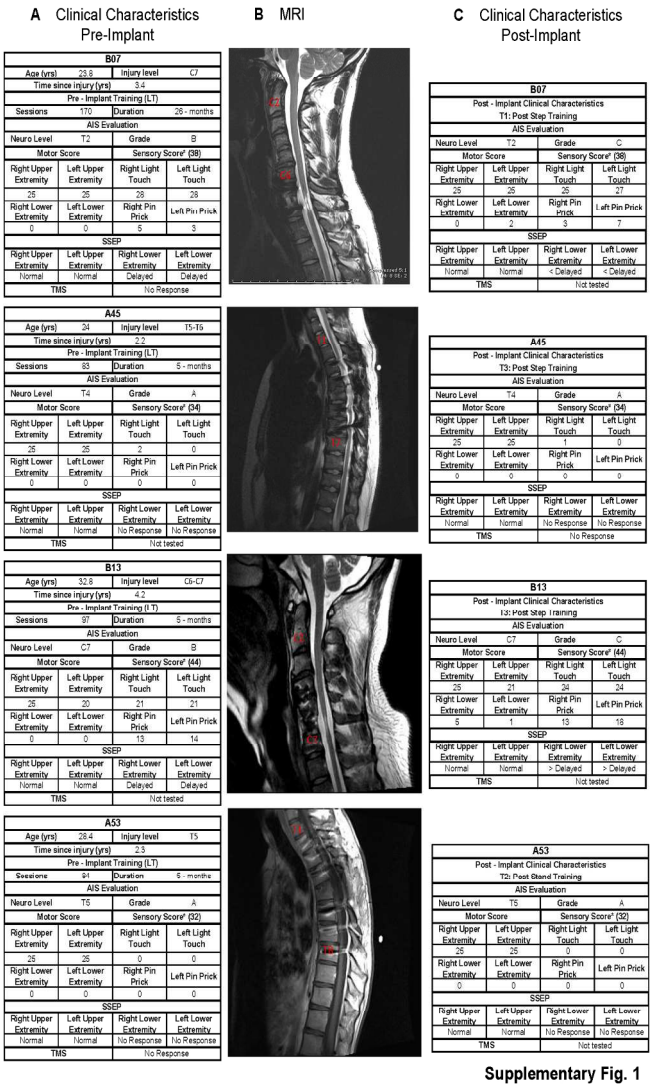

Figure 1: Clinical characteristics for each participant prior to implantation (A) and MRI of injury site for each participant (B). Clinical characteristics for each participant post training with epidural stimulation. * ASIA sensory scores are reported for values below the neurological level of injury. Number is parenthesis represents the highest possible score based on injury level..
215x279mm (200 x 236 DPI)

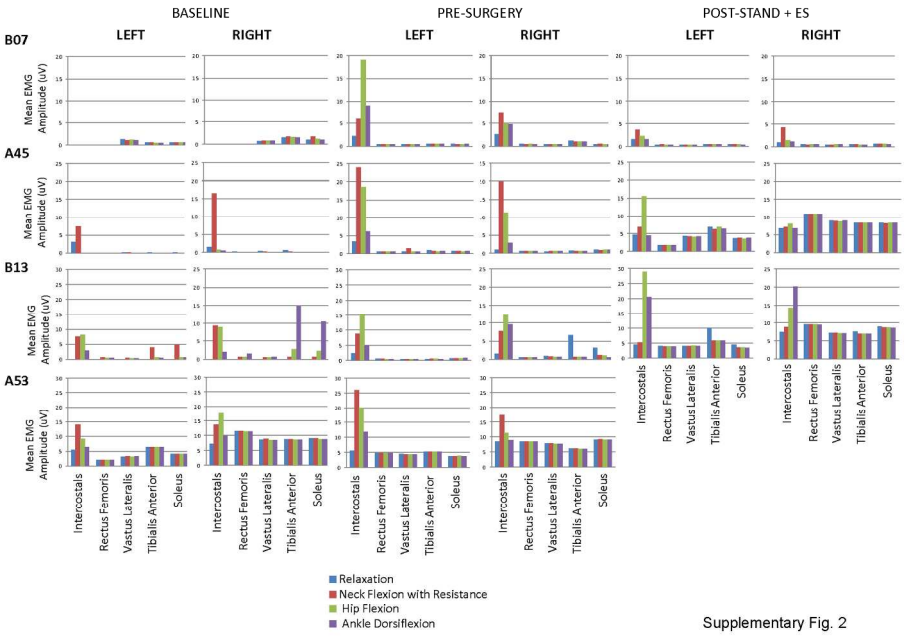

Figure 2: Bar graphs of activity recorded during functional neurophysiological assessments for all subjects during three time points. Selected muscles during four selected tasks are shown. Five-minute relaxation (blue) represents the baseline activity, this serves as a comparison for other tasks. Neck Flexion with Resistance was selected as a reimforcement maneuver while hip flexion and ankle dorsiflexion are representative of active motor tasks.
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Supplementary Materials:

Supplementary Text

Stand Training Description

Stand training occurs over 80 sessions on a custom designed standing apparatus. The research participants attempt to stand for 60 minutes during each training session. To optimize independent standing, stimulation parameters (electrode configuration, voltage and frequency) were modified approximately once per week.

During sitting, the stimulation voltage was increased to the desired level. This voltage was kept constant as the participants go from sit to stand and throughout the standing bout. The research participants initiate the sit to stand transition by positioning feet shoulder width apart and shifting their weight forward to begin loading the legs. The research participants use the bars of the standing device during the transition phase to balance and to partially pull themselves into a standing position. Trainers positioned at the pelvis and knees assist as needed during the sit to stand transition. Elastic bungees posterior to the pelvis are set by one of the trainers after the subject achieves full-weight bearing standing. These bungees help the individual sustain appropriate pelvic tilt and position and allows them to safely stand with minimal assistance. Some participants progress to standing without the posterior bungee.

During the standing bout, one trainer assists the individuals by applying posteriorly directed gentle pressure at the patellar tendon as necessary to maintain knee extension. The research participants were encouraged to stand for as long as possible throughout the session.

Seated resting periods occurred when requested by the research participants and reduced in frequency and duration as the training progressed. No stimulation was provided during the rest periods.

Table S1. Voluntary movement with epidural stimulation experimental sessions

	Figure ~~1~~3		**Figure ~~2~~4**		**Figure ~~3~~5**	
	Experimental Session Number*	Time point (Sup Fig.3)	Experimental Session Number*	Time point (Sup Fig.3)	Experimental Session Number*	Time point (Sup Fig.3)
B07	23	Post stand+ES	29	T1	29	T1
A45	11	T1	11	T1	11	T1
B13	4	T1	--	--	--	--
A53	7	T1	7	T1	7	T1
	Figure ~~4~~6		**Figure ~~5~~7**		**Figure ~~6~~8**	
	Experimental Session Number*	Time point (Sup Fig.3)	Experimental Session Number*	Time point (Sup Fig.3)	Experimental Session Number*	Time point (Sup Fig.3)
B07	44	T3	29, 41, 44	T1-3	--	--
A45	38	T3	11, 25, 30	T1-3	38	T3
B13	28	T2	6,27,35	T1-3	--	--
A53	7	T1		T1	--	--

* Experimental session numbers include all recording sessions performed with epidural stimulation. This can include supine mapping, supine voluntary, standing, and stepping.

Table S2. Table of stimulation configurations used in each figure: cathode gray, anode black, non-active white.

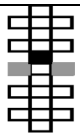
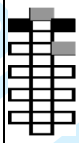
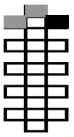
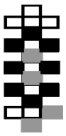
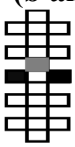
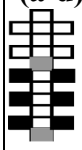
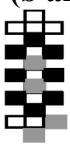
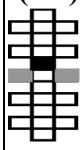
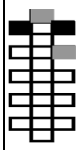
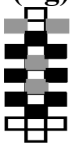
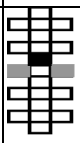
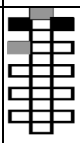
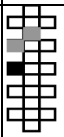
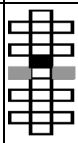
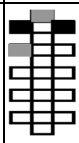
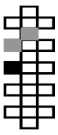
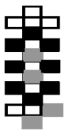
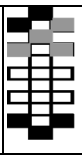
	B07	A45	B13	A53
Frequency **Pulse** **Width**	30 Hz 450 µs	25 Hz 450 µs	30 Hz 450 µs	30 Hz 450 µs
Figure ~~1~~3	 4.0V *210 µs	 5.2V	 0.8V	 2.5V
Figure ~~2~~4	**(b and d)**  4.5V	**(a-d)**  5.3V		**(b and d)**  3.5V
Figure ~~3~~5	**(a-c)**  5.0V	**(d-e)**  7.5V		**(f-g)**  4.4V
Figure ~~4~~6	 5.5V	 5.8V	 0.5V	 3.5V
Figure ~~5~~7	 **(b)** T1: 9.0V T2: 5.9V T3: 5.5V	 **(b)** T1: 5.3V T2: 6.2V T3: 6.4V	 **(b)** T1: 1.5V T2: 0.0V T3: 0.0V	 **(b)** T1: 3.5V T2: 6.7V

Figure ~~68~~		 6.4V; 40 Hz		

* Changes in pulse width

Table S3. Time line of training

Subject ID	Post Implant Training			
	Type	Sessions	Post Implant	
			Start	End
B07	Stand + ES	80	1-mo	6-mo
	Step + ES	80	8-mo	12-mo
	Voluntary + ES	daily	8-mo	ongoing
	Stand + ES (HBP)	daily	12-mo	ongoing
A45	Stand + ES	80	1-mo	6-mo
	Step + ES	80	8-mo	12-mo
	Voluntary + ES	daily	1-mo	ongoing
	Stand + ES (HBP)	daily	12-mo	ongoing
B13	Stand + ES	80	1.6-mo	7-mo
	Step + ES	80	8-mo	12- mo
	Voluntary + ES	daily	1-mo	6 -mo
A53	Stand + ES	80	1.1-mo	7-mo
	Step + ES	75	8-mo	ongoing
	Voluntary + ES	daily	1-mo	ongoing

*sessions are ongoing

HBP: Home based program

Supplementary Figure Legends

Figure 1: Clinical characteristics for each participant prior to implantation (A) and MRI of injury site for each participant (B). Clinical characteristics for each participant post training with epidural stimulation. * ASIA sensory scores are reported for values below the neurological level of injury. Number is parenthesis represents the highest possible score based on injury level.

Figure 2: **Bar graphs of activity recorded during functional neurophysiological assessments for all subjects during three time points.** Selected muscles during four selected tasks are shown. Five-minute relaxation (blue) represents the baseline activity, this serves as a comparison for other tasks. Neck Flexion with Resistance was selected as a reimforcement maneuver while hip flexion and ankle dorsiflexion are representative of active motor tasks.

Supplementary Movie Legends

Movie 1: Right toe movement (flexion/extension) during a training session (non-experimental session). A45 performs three repetitions of movement of first toe with epidural stimulation.

Movie 2: Right ankle movement (plantar/dorsi flexion) during a training session (non-experimental session). A45 performs three repetitions of movement of ankle joint with epidural stimulation.

Movie 3: Right leg movement (flexion/extension) during a training session (non-experimental session). A45 performs three repetitions of movement of hip/knee joints with epidural stimulation.

Movie 4: Repetitive fast leg movements (flexion/extension) during a A45's first experimental session (T1). A non-elastic cable attached to a force transducer measures the force generated during each repetition. The cable is attached to the ankle via a strap.