Original Article

Stepping-like movements in humans with complete spinal cord injury induced by epidural stimulation of the lumbar cord: electromyographic study of compound muscle action potentials

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Study design: It has been previously demonstrated that sustained nonpatterned electric stimulation of the posterior lumbar spinal cord from the epidural space can induce stepping-like movements in subjects with chronic, complete spinal cord injury. In the present paper, we explore physiologically related components of electromyographic (EMG) recordings during the induced stepping-like activity.

Objectives: To examine mechanisms underlying the stepping-like movements activated by electrical epidural stimulation of posterior lumbar cord structures.

Materials and methods: The study is based on the assessment of epidural stimulation to control spasticity by simultaneous recordings of the electromyographic activity of quadriceps, hamstrings, tibialis anterior, and triceps surae. We examined induced muscle responses to stimulation frequencies of 2.2–50 Hz in 10 subjects classified as having a motor complete spinal cord injury (ASIA A and B). We evaluated stimulus-triggered time windows 50 ms in length from the original EMG traces. Stimulus-evoked compound muscle action potentials (CMAPs) were analyzed with reference to latency, amplitude, and shape.

Results: Epidural stimulation of the posterior lumbosacral cord recruited lower limb muscles in a segmental-selective way, which was characteristic for posterior root stimulation. A 2.2 Hz stimulation elicited stimulus-coupled CMAPs of short latency which were approximately half that of phasic stretch reflex latencies for the respective muscle groups. EMG amplitudes were stimulus-strength dependent. Stimulation at 5–15 and 25–50 Hz elicited sustained tonic and rhythmic activity, respectively, and initiated lower limb extension or stepping-like movements representing different levels of muscle synergies. All EMG responses, even during burst-style phases were composed of separate stimulus-triggered CMAPs with characteristic amplitude modulations. During burst-style phases, a significant increase of CMAP latencies by about 10 ms was observed.

Conclusion: The muscle activity evoked by epidural lumbar cord stimulation as described in the present study was initiated within the posterior roots. These posterior roots muscle reflex responses (PRMRRs) to 2.2 Hz stimulation were routed through monosynaptic pathways. Sustained stimulation at 5–50 Hz engaged central spinal PRMRR components. We propose that repeated volleys delivered to the lumbar cord via the posterior roots can effectively modify the central state of spinal circuits by temporarily combining them into functional units generating integrated motor behavior of sustained extension and rhythmic flexion/extension movements. This study opens the possibility for developing neuroprostheses for activation of inherent spinal networks involved in generating functional synergistic movements using a single electrode implanted in a localized and stable region.

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Introduction

There is definite evidence that the spinal cord of lower vertebrates has autonomous capabilities to produce basic coordinated patterns of locomotion, whether in swimming of fish or walking of terrestrial animals in the absence of input from higher levels of the central nervous system or from peripheral feedback.^{1,2} The existence of spinal stepping generators in humans is more difficult to demonstrate and evidence is, by necessity, indirect.³ Bussel *et al*⁴ discussed that some elements of the spinal circuitry on which the generation of stepping rhythms relies in lower vertebrates also exist in man.⁵ Calancie *et al*⁶ investigated involuntary stepping-like movements in a subject with chronic incomplete spinal cord injury (SCI). They concluded that the automatic movement patterns were affected by a preserved but extremely limited supraspinal facilitation and an abnormal afferent inflow from the subject's hip, where they found evidence of pathology. Gurfinkel *et al*⁷ used tonic peripheral afferent stimulation to elicit involuntary stepping in nonparaplegic individuals. They found that continuous lower limb muscle vibration gave rise to involuntary locomotion-like movements in suspended legs and suggested that the nonspecific afferent input induced by vibration activated central structures governing stepping movements.

When epidural spinal cord stimulation (SCS) became a clinical method to control severe spasticity in chronic spinal cord injured individuals,^{8–11} it provided a neurophysiological technique to deliver tonic input to the human spinal cord deprived of supraspinal influence, and to examine locomotor capabilities of the lumbar cord.^{12,13}

In a clinical program of restorative neurology, we evaluated the optimal site and parameters of SCS for control of spasticity and thereby applied stimulation strengths from 1 to 10 V at frequencies from 2.2 to 100 Hz and tested different contact combinations of an epidurally placed electrode array.¹¹ In the course of the evaluation procedure, we discovered that the electrical stimulation of the posterior structures of the lumbar spinal cord could initiate and maintain rhythmic stepping-like flexion/extension movements of the subject's paralyzed limbs. To illustrate our findings in this initial study, we chose recorded electromyographic (EMG) activity of agonist–antagonist muscles of thigh and leg that lasted for 30 s without changes in the rhythmical patterns.¹⁴

In the present paper, we investigate the nature of these responses. We sought to reveal whether the EMG patterns recorded during induced stepping-like activity could be decomposed into physiologically related components. To this end, we extended the time scales of the analyzed EMG traces and examined stimulus-

triggered time windows of 50 ms length. Responses to 2.2 Hz stimulation and pairs of stimuli were evaluated to identify which directly stimulated neural structures should be considered as inputs resulting in the recorded EMG activity. Responses to trains of electrical stimuli of 5–50 Hz were examined to learn how different pulse frequencies cause lumbar cord neurons to shape different types of sustained tonic or patterned rhythmical motor output.

We provide evidence that the flexion/extension movements evoked by epidural lumbar cord stimulation are initiated by immediate stimulation of the posterior roots. It will be demonstrated that during the different induced EMG patterns and even during burst-style phases, each pulse within the stimulus train triggered a separate compound muscle action potential (CMAP). The electrophysiological characteristics of these posterior roots muscle reflex responses (PRMRRs) will be described.

We propose that lumbar interneuronal systems can respond to particular repetition rates of tonic afferent input by temporarily combining into functional networks, which modulate the transmitting pathways and amplitudes of the PRMRRs and thereby generate flexion/extension movements.

Materials and methods

Subjects

The retrospective analyses performed in this study are based on data collected while routinely conducting a clinical protocol for the evaluation of the optimal site and parameters of epidural SCS for spasticity control in subjects who were resistant to other treatment modalities. The effect of stimulation had been assessed by EMG recordings of muscle activity in the lower limbs. For the present study, we selected recordings obtained in 10 subjects who were neurologically classified as having a complete spinal cord lesion at the cervical or thoracic level with no motor functions below the lesion (ASIA A or B). Pertinent patient-related data are listed in Table 1.

At the time of data collection, the subjects met the following criteria: (1) they were healthy adults with closed, post-traumatic spinal cord lesions; (2) all patients were in a chronic (more than 1 year postonset) and stable condition; (3) no antispastic medication was being used; (4) the stretch and cutaneomuscular reflexes were preserved; (5) there was no voluntary activation of motor units below the level of the lesion as confirmed by brain motor control assessment;¹⁵ while (6) surface recorded lumbosacral evoked potentials – used to assess the functions of the posterior structures and gray matter

Table 1 Demographic and clinical data

Subject no.	Sex	Born in	Accident in	Implantation of electrode in	Type of accident	Level of SCI	ASIA class
1	M	1977	1994	1999	Motorbike accident	C6	A
2	M	1973	1995	1998	Car accident	C4	A
3	M	1981	1996	1999	Car accident	C4	A
4	M	1973	1997	1998	Ski accident	C7	B
5	M	1978	1996	1999	Car accident	T10	B
6	F	1978	1994	1996	Car accident	T4	A
7	F	1975	1996	2000	Car accident	T6	A
8	M	1973	1996	1997	Car accident	T4	A
9	F	1965	1996	1998	Car accident	T5	A
10	M	1939	1994	1997	Fall accident	T7	A

M = male; F = female; SCI = spinal cord injury

of the spinal cord below the level of the lesion – were present.^{16,17}

To control their spasticity, all subjects had an epidural electrode array implanted at some vertebral level ranging from T10 down to L1 (see ‘Stimulation and recording setup’). The position of the epidurally placed electrodes relative to the vertebral bodies was obtained from postoperative X-rays. The implantations as well as the clinical protocol to evaluate the optimal stimulation parameters were approved by the local ethics committee. All subjects gave their informed consent.

Stimulation and recording setup

Figure 1a illustrates the patient set-up used, according to the clinical protocol, for the evaluation of the optimal stimulation parameters for spasticity control. Stimulation was delivered via a quadripolar electrode array with cylindrical electrode design (PISCES-QUAD electrode, Model 3487A, MEDTRONIC, Minneapolis, MN, USA) placed in the dorsomedial epidural space at vertebral levels ranging from T10 to L1 (left side of Figure 1a). The data analyzed in the present study was recorded when the electrode array was operated as a bipolar electrode, the most rostral and caudal electrode contacts being connected to the positive and negative outputs, respectively, of the implanted pulse generator (ITREL 3, Model 7425, MEDTRONIC). The separation of the two active electrode contacts was 27 mm. The bipolar impedance was generally about 1000 Ω . The stimulus pulses were biphasic and actively charge balanced. The first dominant phase was about rectangular with a width of 210 μ s, the amplitude in the second phase was small and irrelevant to the stimulation process. Thus, stimulation was virtually monophasic and the stimulating effect of the epidural electrode was based on its polarity during the first dominant phase. Cathodal stimulation of spinal neural structures is known to result in lower thresholds than anodal stimulation. For posterior root fibers, calculated thresholds for cathodal excitation are about three times lower than anodal thresholds.^{18,19} Reversing the polarity of the bipolar epidural electrode shifted the effective

cathode site to a different rostrocaudal level. Thus, setting the electrode polarity allowed for stimulation of posterior structures of the spinal cord at different segmental levels with a single epidural electrode array – given by the cathode site. The maximum stimulation strength was 10 V.

To verify the effect of SCS, EMG activity of quadriceps, hamstring, tibialis anterior, and triceps surae was recorded with silver–silver chloride surface electrodes (right side of Figure 1a). Additional surface electrodes were placed over the lumbar paraspinal trunk muscles. Stimulus artifacts captured by this recording electrode allowed the identification of the onsets of applied voltage pulses. The bipolar surface electrodes were placed centrally over the muscle bellies spaced 3 cm apart and oriented along the long axis of the muscles. The skin was slightly abraded such that an electrode impedance of less than 5 k Ω was reached. The EMG signals were amplified with the Grass 12D-16-OS NEURODATA ACQUISITION SYSTEM (GRASS INSTRUMENTS, Quincy, MA, USA) adjusted to a gain of 2000 over a bandwidth of 30–1000 Hz and digitized at 2048 samples/s/channel using a CODAS ADC system (DATAQ INSTRUMENTS, Akron, OH, USA). The EMG data was analyzed off-line using WINDAQ Waveform Browser playback software (DATAQ INSTRUMENTS).

One of two different sensor types was used to record knee movement, namely goniometers (Model XM-180, and K100 AMPLIFIER SYSTEM, PENNY & GILES BIOMETRICS LTD.) or electronic clinometers (ACCUSTAR, LUCAS SENSING SYSTEMS, Phoenix, AZ, USA) both attached bilaterally to the knee. The goniometer measured relative changes in the knee joint angle as illustrated in the left side of Figure 1b. The clinometer measured rotations about its axis and thus rotations of the longitudinal axis of the thigh around the hip.

All recordings were conducted with the subjects placed on a comfortable examination table covered with soft sheepskin in a supine position. This configuration allowed flexion/extension movements of the lower limbs to unfold smoothly and minimized friction between the heel and the supporting surface.

Figure 1 Outline of the clinical assessment design and analysis of stimulus-evoked EMG responses. (a) All recordings were conducted with the patients in a comfortable supine position. Pairs of surface EMG electrodes were placed over the bellies of the lower limb muscle groups to assess the effects of epidural stimulation. (b) To monitor relative movements of the lower limbs, goniometers or clinometers were applied bilaterally to the knee. (c) EMG responses of triceps surae to epidural stimulation at 2.2 Hz (top) and 22 Hz (bottom) displayed with different time scales. Potentials marked by gray backgrounds are shown in extended time scale ($\times 10$) on the right side of the original EMG. Traces of EMG responses (10 s) are displayed in the left column. In the middle column, it is shown that stimulus artifacts (recorded by paraspinal-surface electrodes) allow the identification of the onsets of applied voltage pulses. In the right column, EMG potentials of separate responses can be seen. These CMAPs were analyzed for latencies, peak-to-peak amplitudes, and shapes irrespective of the stimulation amplitude or frequency

Data analysis – data based on incremental pulse amplitudes

The first set of EMG data had originally been collected to define the rostrocaudal level of the epidural electrode relative to the lumbosacral cord segments based on muscle twitch recruitment patterns.²⁰ For this purpose, a frequency of 2.2 Hz was used, and the stimulation strength was intensified in 1-V increments from 1 to 10 V.

In the present study, we examined the relationship between the rostrocaudal position of the cathode relative to the spinal cord and the sequence of quadriceps and triceps surae activation. The vertebral cathode level was obtained from X-ray. The estimated, functional segmental level of the cathode was derived from a neurophysiological technique for electrode positioning in subjects with impaired sensory function.²⁰ Additionally, information on average spatial relations

between the cord segments and the vertebral bodies was used.²¹ The effect of two different rostrocaudal cathode sites were studied in all subjects but except subject 4. The electrode array of subject 4 was repositioned a number of times during participation in the clinical program. This allowed comparison of stimulation with six different cathode sites located between the upper third of the T10 and the upper L1 vertebra in a single subject.

The recruitment sequence of quadriceps and triceps surae was given by the whole-numbered threshold voltages for the respective muscles. Only quadriceps and triceps surae were considered in this analysis, because they are muscle groups with separate segmental innervations (L2–L4 versus L5–S2).

We examined single stimulus-evoked and surface-recorded CMAPs for latencies (quantitatively), peak-to-peak amplitudes, and shapes (qualitatively) of the same data set. The latencies were read off-line from the EMG traces using the playback software and measured as the time between stimulation onset – identified by stimulus artifacts – and the first deflection of the EMG potential from baseline (Figure 1c, top). The mean latency times based on all subjects were calculated for the threshold stimulus strengths, eliciting responses in each muscle group and for the maximum strengths applied.

Data analysis – data based on incremental pulse frequencies

The second set of EMG data that we analyzed had originally been collected to assess the effect of trains of 5–100 Hz stimuli on spasticity.¹¹ These data were used to evaluate the refractory periods of responses to pairs of stimuli delivered at intervals of 200–10 ms, based on the first two stimuli of the applied trains.

Furthermore, we examined the muscle activity induced by sustained trains of 1–10 V and 5–50 Hz, focusing on the effects of different repetition rates of stimulation on the evoked motor output patterns. To reveal the components of the overall EMG pattern, we subsequently enlarged the time scale of the EMG traces. We addressed the question of how far individual responses to single pulses within the applied stimulus train were reflected in the EMG output pattern. Separate successive EMG responses were analyzed for latencies (quantitatively), peak-to-peak amplitudes, and shapes (qualitatively) (Figure 1c, bottom).

Results

Epidurally evoked segmental twitch responses

Electrical stimulation of the lumbosacral cord delivered from the dorsomedial epidural space at 2.2 Hz and 1–10 V gave rise to stimulus-coupled CMAPs in the lower limb muscles. Figure 2a shows successively elicited single CMAPs of quadriceps and triceps surae muscles, induced by stimulation with 3–7 V and 2.2 Hz (subject 3). The effective cathode was located at the center level of the T12 vertebral body, the estimated segmental level was L3/L4. Stimulus-triggered time windows 50 ms in

length were extracted from the original continuous EMG traces (see also Figure 1c). The left margins of the windows are determined by the onsets of the stimuli. EMG responses to 10 consecutive stimuli are shown for each incremental voltage. In the presented case, CMAPs were not induced in the lower limb muscle groups at SCS strengths of 1–2 V. At 3 V, CMAPs with short (9.5 ms) and constant latencies were recorded from quadriceps, while triceps surae showed no activity. At 4 V, quadriceps showed higher CMAP amplitudes than at 3 V while the latencies remained unchanged. Faint activity was also observed for triceps surae at 4 V, involving latencies of 18.5 ms. At higher stimulation strengths, CMAP amplitudes further increased for both muscle groups. The latencies of successive responses remained constant, while no additional components with longer latencies emerged in the EMG traces at higher stimulus strengths. For a given stimulus strength, amplitudes of successive CMAP showed only minor and unsystematic variations. The demonstrated muscle recruitment sequence was representative for cathodes positioned at the level of the T12 vertebral body.

As shown in Figure 2b, this recruitment pattern was essentially reversed when the effective cathode was located at the lower third of the L1 vertebral body, corresponding to the average position of the conus medullaris. A total of 10 CMAPs are displayed sequentially for incremental stimulus strengths of 4–8 V (subject 5). At the threshold level of 4 V, CMAPs with a latency of 18.5 ms and rather large amplitudes were evoked in triceps surae. The CMAP amplitudes showed progressive increases with increasing stimulus strength until a plateau of EMG activity was reached at 7 V. Weak quadriceps responses with a latency of 10.5 ms were initiated at a strength of 5 V. Quadriceps CMAPs with only moderate amplitudes were evoked even at increased stimulus strength.

Figure 2 demonstrates a strong relationship between the rostrocaudal position of the cathode relative to the spinal cord and the sequence of quadriceps and triceps surae activation. In subjects with cathodes located at the T11 vertebral level, rather strong stimulation amplitudes (6–9 V) were needed to elicit CMAPs in the quadriceps muscle, while triceps surae could not be activated even at the maximum strength of 10 V (subjects 4, 6, 9). Note that on average, the T11 vertebral level corresponds to the L1 and L2 segmental levels, while the levels L5–S2 are situated well below this rostrocaudal position. The lower third of the T10 vertebral body was the most rostral position from which lower limb muscle responses could be evoked at a stimulation strength of 10 V – in fact solely in the thigh muscles. From the upper third of the T10 vertebral body, none of the studied lower limb muscles could be activated with strengths up to 10 V, although the lumbar paraspinal trunk muscles responded at a threshold of 5 V (subject 4).

EMG data derived from subject 4 during epidural stimulation with considerably different rostrocaudal positions of the effective cathode (see Materials and

Figure 2 EMG responses to 2.2 Hz stimulation. Stimulus-triggered raster presentation of CMAPs induced in quadriceps and triceps surae. The first 50 ms following each stimulus pulse are shown. (a) The effective cathode was located at the center level of the T12 vertebral body, which approximately corresponds to L3/L4 segmental level (subject 3). The stimulation strength was increased in steps of 1 V. In total, 10 single EMG potentials of successive muscle responses are shown for each incremental voltage (3–7 V). (b) Effective cathode site was at the lower level of the L1 vertebra, corresponding to the level of the conus medullaris (subject 5). A total of 10 stimulus-evoked CMAPs are displayed for each incremental voltage (4–8 V)

methods) allowed us to reveal any relationship between the segmental cathode level and the response latencies. When the cathode was at the lower third of the T10 vertebral body, CMAPs elicited in quadriceps and hamstrings at threshold level had mean latencies of 9.8 and 10.3 ms, respectively. When the cathode was repositioned caudally by 7 cm to the upper L1 vertebral level, the latencies were 9.5 and 10.0 ms. Considering that the accuracy of identification of the latencies was 0.25 ms at best, no significant correlation between rostrocaudal cathode position and response latencies could be demonstrated. Therefore, we calculated the average response latencies from the data derived from all subjects irrespective of the positions of their epidurally placed electrodes for quadriceps, hamstrings, tibialis anterior, and triceps surae for 2.2 Hz stimulation. The mean latency times for these muscle groups at threshold level and maximum applied stimulation strength (generally 8–10 V) are compiled in Table 2 and were about 9–10 ms for the thigh muscles and 16–17 ms for the distal leg muscles. Note that the weak responses induced at threshold stimulation had low CMAP amplitudes and slight onset slopes of the potentials, thus making the precise identification of the onset of the CMAPs difficult. This may account for

the longer latencies of muscle responses at threshold level in Table 2.

To summarize, 2.2 Hz stimulation of the posterior structures of the lumbosacral cord elicited twitch responses in the lower limb muscles in a segmental-selective way (Figure 2). The EMG signals associated with these responses were stimulus coupled and of short latency (Table 2) – approximately half that of the phasic stretch reflexes of the respective muscle groups. Equal-voltage stimuli yielded CMAPs with negligible variations of amplitudes and shapes. Thus, a given response demonstrated no influence on following responses to the stimulus train. Responses of a muscle were not influenced by the ongoing activity of responses of the other muscles to the same stimulus pulse. For a given cathode position, the CMAP amplitudes elicited at 2.2 Hz were dependent on the stimulus strength.

Stimulation with pairs of stimuli

While similar stimuli at 2.2 Hz yielded EMG responses with similar amplitudes (Figure 2), different patterns emerged when pulses were applied in close succession. First we analyzed CMAPs induced by pairs of stimuli of

equal strength delivered to the posterior structures of the lumbosacral cord at different intervals.

Figure 3 shows a representative example of responses to pairs of stimuli with different interstimulus intervals (ISIs) derived from the quadriceps muscle (subject 1). Arrows mark the onsets of stimulus pulses. The stimulus strength of the pairs of stimuli was increased in steps of 1 V from threshold level (4 V, top row) to 50% higher voltages (6 V, bottom row). The first stimulus evoked a pronounced CMAP regardless of the interstimulus intervals and can be considered as a control response. At 4 V, the second stimulus applied after an interstimulus time of 56 ms only evoked a weak response. The CMAP amplitude of this test response was 7% of the control response magnitude. At the same interstimulus time, but higher strengths of epidural stimulation, the second stimulus was capable of inducing a CMAP with an amplitude nearly as large as the first one. At a

stimulus strength of 4 V and an ISI of 20 ms, the second pulse completely failed to elicit a muscle response, while a second response occurred again when the stimulus voltage was increased.

Thus, the refractory behavior of epidurally evoked muscle responses to pairs of stimuli did not only depend on the ISI but also on the stimulation strength. At threshold level, we found long refractory periods of up to 47.5 ms (subject 1) and even 62.5 ms (subject 3). The shorter the ISIs were, the lower were the test response magnitudes, with 20 ms being the earliest point for the second stimulus to result in unequivocal CMAPs. At intervals shorter than 20 ms, the presence of the final deep positive potential of the first CMAP introduced difficulties in interpreting the decrease in amplitude of the second response.

Figure 3 demonstrated that a single stimulus pulse and the corresponding response has long-lasting, stimulus-strength-dependent conditioning effects on the excitability of the activated structures.

Table 2 Mean latencies (ms±SD) of CMAPs evoked by 2.2 Hz stimulation based on all subjects at threshold and maximum stimulation strength

	Threshold	Maximum
Q	9.3±1.6	8.6±0.9
H	9.9±1.3	9.3±0.8
TA	16.4±2.1	16.1±1.6
TS	16.7±1.5	16.3±1.4

Stimulation with trains of stimuli

Figure 4a displays EMG recordings and goniometer traces derived from subject 7 during stimulation at 6 Hz (i) and 31 Hz (ii) without departing from the same sustained and nonpatterned mode of input application to the same spinal cord level. Stimulation strength was 10 V, the estimated functional segmental level of the cathode was L3/L4. At the onset of the

Figure 3 EMG potentials of quadriceps (subject 1) induced by pairs of stimuli delivered at different intervals and stimulation strengths. The arrows indicate the onset of each stimulus pulse. ISIs of 56 ms (18 Hz), 40 ms (25 Hz), and 20 ms (50 Hz) were tested. Stimulation strength was increased from threshold level (4 V) to 150% of the threshold (6 V) in steps of 1 V. The percentage value in each box gives the EMG amplitude of the second response relative to the first response

Figure 4 EMG responses to trains of stimuli showing characteristic modulations of CMAP amplitudes. **(a)** EMG recordings obtained from quadriceps (Q), hamstrings (H), tibialis anterior (TA), and triceps surae (TS) during SCS at frequencies of 6 Hz (i) and 31 Hz (ii). The goniometer traces (KJA – knee joint angle) illustrate the corresponding induced extension and rhythmical movements, respectively, of the lower limbs. The position of the cathode (estimated functional segmental level: L3/L4) and the stimulation strength (10 V) were left unchanged when switching frequencies. **(b)** Surface EMG recordings from the right quadriceps (Q), hamstrings (H), tibialis anterior (TA), and triceps surae (TS); position sensor trace demonstrating flexion/extension movements of the knee (KM). The estimated functional segmental cathode level was at L4/L5. Stimulation parameters were 9 V and 30 Hz (subject 8). The first burst-style phase of quadriceps marked by gray background is displayed in extended time scale on the bottom of the original EMG along with stimulus artefacts captured by electrodes placed over the lumbar paraspinal trunk muscles

recordings shown on the left side, the subject's lower extremities had been passively moved to the point of maximum possible flexion, and stimulation was subsequently applied at 6 Hz. The sustained stimulation initiated and maintained an extension movement of the lower limbs. The recruited muscles were visibly contracting briefly, with the tonic EMG output in hamstrings and triceps surae being greater than in their antagonists. During the actual extension move-

ment, unfolding from the initially flexed position of the lower limbs, the EMG pattern revealed well-defined temporal modulations that were directed toward knee joint stabilization. Thereby the EMG activity in hamstrings progressively decreased while the one in quadriceps increased. When the end point of the movement was reached, and the stimulation was sustained, the limbs remained in the extended position, with the muscles visibly contracting. When the electrical

stimulation was turned off, the lower limb muscles relaxed immediately.

We studied this finding that epidural stimulation of the human lumbar cord isolated from brain control can induce a sustained extension of the lower extremities in more detail in five of our subjects (subjects 2, 4, 6, 7, 9). Stimulation at 5–15 Hz and 6–10 V applied to the lumbar cord reproducibly elicited the characteristic temporal pattern of EMG-amplitude relations between antagonistic muscles and led to lower limb extension in all five subjects. The induced extension pattern was observed in different trials. The consistency of this finding only depended on application of the appropriate stimulus parameters.

On the right side of the Figure 4a it is demonstrated that by increasing the frequency of the stimulus train to 31 Hz, the previous modulated-tonic EMG activity was replaced by a rhythmical one (ii). The EMG recording revealed alternating phases of burst-style activity in the recruited lower limb muscles. While this is not the best example of an induced stepping-like activity (coactivation of TA and TS), both recordings (i) and (ii) were made during a single session in the same subject without changing the site or strength of stimulation. From this observation it was clear that the spinal cord isolated from supraspinal input was capable of shaping both a motor output with extensor muscles dominating over the flexors and rhythmical reciprocal activation of flexors and extensors in response to sustained stimulation. The stimulation frequency determined which type of induced motor activity was established.

The finding that epidural lumbar cord stimulation can generate stepping-like EMG activity was studied in our pool of subjects. Stepping-like, alternating flexion/extension movements in the lower limbs were repetitively initiated in different recording sessions, which were up to several months apart in a given subject and also in different subjects. Optimal parameter settings were 25–50 Hz and 6–10 V. The cathode site had to provide a dominant stimulation of the posterior roots entering the upper lumbar cord. Lower thresholds for recruitment of quadriceps than of triceps surae characterized such a location (see Figure 2a). Moreover, strengths had to be above the level for eliciting CMAPs in quadriceps as well as triceps surae for a given cathode location. Stimulation commonly induced unilateral stepping-like flexion/extension movements. Similarly, in about 2/3 of all analyzed cases in our subject pool, a unilateral muscle twitch distribution at threshold stimulation level was observed, indicating asymmetric position of the epidural electrode array relative to the spinal cord. Stepping-like movements of the side associated with the lower thresholds were induced. The contralateral lower limb responded either with tonic activity or with synchronous burst-style coactivations of all studied muscles of this limb.

During 2 s of recording shown in Figure 4a (i), 12 separate CMAPs in each muscle were elicited (see the time marker). Thus, a single CMAP was elicited by each stimulus pulse of the 6 Hz train which induced the

characteristic EMG-extension pattern. These consecutively elicited separate CMAPs resulted in the small deflections superimposed on the overall trace of the goniometer recording in Figure 4a (i). Figure 4b presents surface EMG recordings from the right quadriceps (Q), hamstrings (H), tibialis anterior (TA), and triceps surae (TS) and position sensor recordings showing knee flexion/extension movements (subject 8). The rostrocaudal cathode site was at the T12/L1 intervertebral level, the functional segmental level about L4/L5. Stimulation parameters were 9 V and 30 Hz. The EMG traces demonstrate alternating phases of burst-style activity in the lower limb flexor and extensor muscles. The corresponding position sensor trace confirms that the induced muscle activity led to actual stepping-like flexion/extension movements of the lower limb. Deflection up indicates flexion and deflection down indicates extension of the lower limb. The range of knee movement was about 65°.

At the bottom of Figure 4b the first of the burst-style phases of quadriceps is displayed in extended time scale along with corresponding stimulus artefacts derived from the paraspinal muscle, which indicate the onsets of the stimulus pulses. It can be clearly seen that each pulse of the stimulus train triggered a single CMAP. Thus, the burst-style phase consisted of stimulus-triggered, separate CMAPs that were subject to well-defined amplitude modulations resulting in a burst-like shape of the EMG activity. Another example of this finding was demonstrated for triceps surae in Figure 1c (bottom figure).

In Figure 5 we compare the EMG features of single CMAPs in response to stimulation with different repetition rates and during different phases of induced rhythmic motor activity. Figure 5a is a stimulus-triggered sequential presentation of CMAPs induced in tibialis anterior by sustained epidural stimulation (subject 1). All CMAPs were recorded during a single session. Stimulation strength was 5 V and constant when frequency was varied. The functional segmental cathode position was L3/L4 and identical in all cases. In all, 10 successive CMAPs are shown for a given repetition rate. Stimulus trains with frequencies of 2.2, 11, and 16 Hz induced tonic EMG activity, whereas at 22 Hz a pattern with slow rhythmical amplitude modulations was evoked. CMAPs elicited at 2.2 Hz had a short, constant latency of 16 ms and rather similar shapes and amplitudes. CMAPs composing the activity in response to a 10 times higher frequency of stimulation had a longer and fairly constant latency of 23 ms. The early negative and positive potential of the CMAPs induced at 2.2 Hz were absent, while additional EMG components with longer latencies emerged. Analyzing the CMAPs constituting the tonic EMG patterns elicited at 11 and 16 Hz revealed transitional stages between the 'short-latency' response to 2.2 Hz stimulation and the 'long-latency' response to 22 Hz stimulation. This becomes obvious when 10 successively elicited CMAPs were averaged and compared (right side of Figure 5a). By increasing the frequency from 2.2 to 11 Hz, the 'short-latency' response decreased in amplitude while

Figure 5 Changes of reflex pathways due to increased repetition rates of the sustained stimulation and during induced rhythmic EMG activity. **(a)** Stimulus-triggered sequential presentation of CMAPs induced in tibialis anterior by sustained epidural stimulation at a constant strength of 5 V but different frequencies (subject 1). Cathode position was identical in all cases. A total of 10 single EMG potentials of successive muscle responses are shown for a given stimulation frequency. Averaged responses derived from the respective 10 single responses are displayed on the right side of the figure. A template based on the response to 2.2 Hz stimulation is used to indicate changes of the CMAP shape when different frequencies were applied. **(b)** Continuous EMG of tibialis anterior responses showing three burst-style phases induced by stimulation with 7 V and 28 Hz along with the latencies of identified separate CMAPs (subject 4). Three single CMAPs evoked before (#1 and #2) and during (#3) the first burst-style phase are displayed in extended time scale at the bottom of the figure. The left margins of the 50-ms time windows indicate when a stimulus was applied

small-amplitude late potentials were building up. At 16 Hz, the early components of the CMAP were further attenuated and the late components further enhanced. Further decrease of the early components and a dominating contribution of the later ones lead to the CMAP shapes as seen in response to 22 Hz stimulation.

Figure 5b displays a continuous EMG of tibialis anterior responses showing three burst-style phases induced by stimulation with 7 V at 28 Hz (trace with corresponding right scale bar). The data is derived from subject 4. The displayed EMG activity is part of a

recording of an epidurally induced flexion/extension movement of the lower limb. The burst-style phases alternated with phases of small amplitude responses allowing the identification of CMAP latencies during the different phases and the transitions between them. The measured latencies are indicated by black diamonds in the figure and are placed in accordance to the times the corresponding CMAPs were initiated (diagram with left scale bar and same abscissa as the EMG trace). The latencies are shown for every third/fourth single stimulus-evoked CMAP (72 out of 243 successively evoked CMAPs within the displayed 9 s-trace). The

missing data points did not differ from the presented relation. While the CMAP latencies were 15–17 ms during the phases with small amplitude responses, they increased significantly to 25–27 ms during the burst-style phases. Thus, CMAP amplitudes as well as latencies were subject to modulations during induced rhythmic activity. Three single EMG responses are presented in extended time scale showing characteristic CMAP shapes during the different phases of the rhythmic EMG activity at the bottom of the figure. The left margins of the 50-ms time windows indicate when a stimulus was applied. The first CMAP (time marker #1) was elicited between two burst-style phases. It was of small amplitude and had a short latency of 16 ms. The third CMAP (#3) was the one with the largest amplitude during the first burst-style phase, where each pulse of the train elicited a separate CMAP. The displayed CMAP had a latency of 25 ms. Its shape was similar to the tibialis anterior CMAPs in response to 22 Hz depicted in Figure 5a. Since the timeframe covering both the prolonged response latency and the CMAP duration is longer than the interstimulus interval (36 ms), the final negative potential of the immediately preceding response is seen at the beginning of the time window (covering the first 10 ms). The second CMAP (#2) displayed in extended time scale was elicited between the two time markers #1 and #3 and incorporates both the component of the short latency CMAP and also additional later potentials.

We found that the short-latency CMAP as described in Figure 2 was followed by later EMG components or was even completely replaced by a longer latency CMAP when stimulation frequencies of about 16 Hz or more were used. They were observed consistently when rhythmic EMG activity was induced in the lower extremities. Indeed, in different cases of epidurally evoked rhythmic EMG activity, the pattern of CMAP latency prolongation was dissimilar. In some cases only the tibialis anterior muscle showed the longer latency response, in others tibialis anterior and triceps surae, or only the muscle groups functionally acting as flexors showed the longer latency responses. In some cases (subject 7), the stimulus-coupled CMAPs of all recorded lower limb muscles demonstrated prolonged latencies. Analyzing the basis of these differences was beyond the scope of this study. The longer CMAP latencies were prolonged by about 10 ms compared to the CMAPs elicited at 2.2 Hz. An example of a longer latency response induced in the triceps surae muscle was shown in Figure 1c (bottom figure).

To summarize, we have shown that epidural stimulation applied to the same cord segments can evoke two distinct patterns of EMG activity in the lower limb muscles: (i) characteristically modulated tonic EMG activity appropriate to initiate and maintain extension of the lower extremities and (ii) burst-style EMG activity in flexors and extensors that appeared in appropriate sequences to induce rhythmic flexion and extension movements of the lower limbs. Inducing these patterns was a function of different repetition rates of

the applied train of electrical stimuli. Decomposing the EMG 'interference patterns' into their fundamental physiologically related components was possible by extending the time scales of the analyzed EMG sequences. During the different induced EMG patterns and even during burst-style phases, each pulse within the stimulus train triggered a separate CMAP.

Discussion

Directly stimulated structures which should be considered as inputs resulting in the CMAPs

We have shown that epidural stimulation can evoke different patterns of lower limb muscle activation depending on the frequency of the delivered sustained stimulation. At low frequency of 2.2 Hz the amplitudes of stimulus-evoked CMAPs were determined by the stimulus strength. When the lower limbs were passively moved to the point of maximum possible flexion and trains of stimuli were applied at 5–15 Hz, an extension movement was induced and maintained.^{22–24} Successively elicited CMAPs showed characteristic amplitude modulations with a stronger motor output of the extensor muscles than of the flexors. Stimulation at 25–50 Hz induced alternating burst-style EMG activity in flexors and extensors leading to stepping-like movements. Stimulation at even higher frequencies of 50–100 Hz decreased the muscle tone of the lower limbs.¹¹ These patterns could be elicited with a single cathode placed in the posterior aspect of the epidural space over the upper lumbar segmental levels without changes of position (Figure 4a (i) and (ii)). Which neural structures have directly been activated by the electrical stimulation resulting in the described muscle activities?

Direct activation of motoneurons – thus bypassing the spinal cord – can be ruled out because this could not account for the specific amplitude modulation of CMAPs during stimulation with constant parameters as presented in Figures 4 and 5. Moreover, CMAPs studied with pairs of stimuli had long refractory periods of up to 62.5 ms. In contrast, direct activation of ventral root motor fibers or motoneurons in the ventral horn follow a stimulus pulse train up to 100 Hz.^{18,25}

For epidural SCS with dorsal electrode placement fibers within the posterior roots or superficial posterior columns have the lowest thresholds.^{19,26} Therefore, we will discuss whether stimulation of posterior roots or columns may explain the segmental-selective effect of the stimulation as described in Figure 2 and the EMG features of the recorded CMAPs.

The population of axons within the posterior columns at any spinal level arises from the posterior roots of numerous segments, both rostral and caudal to that level. Thus the effect of posterior column stimulation can be expected to be general rather than localized to afferents of any specific segmental origins. Hunter and Ashby²⁶ reported that epidural stimulation of the posterior columns at thoracic cord levels resulted in nonselective activation of motoneurons in thigh and leg

muscles. Further indication that stimulation of posterior column fibers is not segmentally selective comes from the application of SCS in chronic pain management basing on reports of the distribution of paresthesiae of patients with intact sensory functions. There, medially placed electrodes elicit a widespread distribution of paresthesiae in body areas at the spinal level of stimulation and far caudally to it, even at the perception threshold.²⁷ The widespread distribution of paresthesiae is attributed to activation of the posterior column fibers.²⁸

The most rostral cathode levels from which CMAPs as described in the present study could still be induced at maximum pulse intensity (10 V) were T10/T11 vertebral levels for quadriceps and T11/T12 for triceps surae. Guru *et al.*²⁹ and Hunter and Ashby²⁶ reported recruitment of thigh and leg muscles by epidural stimulation of posterior column fibers with cathodes located at positions as rostral as the T8–T10 vertebral levels. At these cathode levels, the cord segments and associated roots innervating the lower limbs are located well below the cathode. The latencies reported in these studies were generally about 6–12 ms longer than those of the comparable muscles compiled in our Table 2.

We conclude that the CMAPs described in the present study are not related to posterior column fiber activation. Thereby, our results do not contradict the findings of Guru *et al.*²⁹ and Hunter and Ashby.²⁶ The differences of the effects of stimulation are most probably due to the different subject profiles, electrode setups and the significant differences in the anatomy of the thoracic and the lumbosacral spinal cord. Owing to the disparity in length between the spinal cord and vertebral column, the spinal roots from lumbar and sacral segments have a much longer distance to travel prior to reaching their respective foramina of exit. Whereas cervical and upper thoracic rootlets and roots are situated nearly perpendicular to the cord, lower thoracic, lumbar, and sacral ones enter and leave the cord at increasingly more oblique angles. Most of the circumference of the lower lumbar and sacral cord is covered by the roots.³⁰ For epidural stimulation of the lumbosacral cord, thresholds of fibers within the cord will presumably be increased due to this poorly conducting 'root-layer'.

We infer that the stimulus-evoked CMAPs as described in our study are reflexively elicited by a dominating input via large afferents within the posterior roots. Geometric and electric factors result in low-threshold sites for electrical stimulation where posterior root fibers enter the spinal cord.^{18,31–35} At these sites, posterior root fibers entering the cord caudal to the cathode can have lower thresholds than longitudinal fibers passing the level of the cathode. The high electric conductivity of the cerebrospinal fluid and the longitudinal orientation of the white matter allow the current to spread in a rostrocaudal direction. In this way, recruitment of posterior root fibers is not limited to the level of the cathode. Computer simulation of the epidural electrode setup as used in the present study showed that the largest lumbosacral posterior root fibers

entering the cord caudally to the level of the cathode could still be activated by a maximum stimulation voltage of 10 V at a distance of up to 2 cm.³³

Depending on the relative rostrocaudal position of the cathode with respect to a given posterior root fiber, we suggest two different sites of the initiation of afferent volleys in the posterior roots. For cathode positions at upper lumbar cord levels, lumbar posterior root fibers entering the cord in the vicinity of the cathode will have lowest thresholds. Increasing the stimulation intensity will result in stronger afferent volleys to upper lumbar cord segments and a current spread in the caudal direction inducing muscle responses via posterior root recruitment at the low-threshold sites distant from the cathode. This mechanism can explain the exemplary result in Figure 2a, which is characteristic for cathodes at L2–L4 cord levels.

For caudal cathode positions at the level of the conus medullaris, all segments with afferent fibers coming from the lower limb muscles are represented by posterior root fibers and are organized in layers, which are separated in a posteromedial-anterolateral direction.³⁰ Considering that at the level of the conus medullaris all posterior root fibers are mainly oriented in similar rostrocaudal direction, different thresholds will be predominantly determined by different cathode-fiber distances (for a given fiber size). Owing to a larger distance of the L2–L4 posterior roots than the S1 and L5 posterior roots to a dorsally positioned cathode,³⁰ it is plausible that stimulation of these structures can explain the reversed recruitment order of the characteristic case shown in Figure 2b.

We propose that the posterior root fibers were also the input structures resulting in the more complex motor output patterns at higher stimulation frequencies. First, the directly stimulated long axons within the posterior roots can easily follow the applied frequencies of 2.2–50 Hz. Secondly, stimulation at higher frequencies also showed the segmentally selective recruitment of muscle responses as presented in Figure 2.¹⁴ Finally, we demonstrated that each single stimulus of the applied train, which induced the characteristic EMG-extension pattern, elicited a single CMAP (Figure 4a (i)). During stimulus-evoked 'burst-style' EMG phases each stimulus was followed by a CMAP (Figure 1c, bottom; Figures 4b and 5b, CMAP #3). Shape and duration of these CMAPs were similar to those evoked at 2.2 Hz.

Epidural stimulation induces PRMRs

Posterior root fibers are the predominant input structures for epidural stimulation at lumbosacral segmental levels. The observed twitch responses to 2.2 Hz stimulation were due to activation of large group-Ia primary spindle afferents within the posterior roots with subsequent recruitment of motoneurons through monosynaptic connections in the spinal cord.^{36,37} This mechanism can explain the short and constant latencies of the recorded muscle responses (Figure 2, Table 2). Thus the muscle responses at 2.2 Hz are the physiological

equivalent of the H-reflex elicited at the peripheral nerve. The essential difference is that the reflex induced by epidural stimulation is initiated at a more proximal site in the posterior roots – hence a short segment of the afferent arc must be traversed to elicit the reflex.

We hereinafter refer to this type of reflex responses arising from a dominating input via large afferents within the posterior roots as PRMRRs. Afferents other than group-Ia primary spindle afferents – like Ib afferents from Golgi tendon organs, cutaneous afferents, or group-II secondary muscle afferents – seem not to contribute to the reflex responses to 2.2 Hz stimulation. This does not mean that some of these afferents are not activated by the stimulation. They might contribute to shape the motor output when stimulated repetitively at higher frequencies. We suggest that the sustained input via the posterior roots not only affects monosynaptic excitatory action on motoneurons but concomitant activation of spinal interneurons by synaptically evoked depolarization. At particular repetition rates of stimulation these activated populations of spinal interneurons exert defined facilitatory and inhibitory influences on various motoneuron pools.

PRMRRs and induced central effects

When pairs of stimuli were applied in close succession, the second response was influenced by the ongoing activity of the first response (Figure 3). This refractory behavior of pairs of epidurally evoked reflex responses depended on the interval between the applied stimuli and also on the stimulation strength. The stimulus-strength-dependent range of the refractory periods of dual PRMRRs (20–62.5 ms) can presumably be ascribed to effects of the first volley activating spinal interneuronal structures and corresponding presynaptic mechanisms involved in the control of the motor output.

Activation of central mechanisms was apparent when sustained trains of 5–100 Hz were used instead of pairs of stimuli. At 25–50 Hz, separate CMAPs were subject to well-defined amplitude modulations resulting in burst-like envelopes of the EMG activity (Figure 1c, bottom; Figure 4a (ii) and b). The sustained stimulation not only activated neural structures transmitting the PRMRRs but also recruited mechanisms involved in a well-coordinated gain control of the PRMRR pathways.

Prolongation of the PRMRR latency was another indication that the sustained epidural stimulation could activate structures other than the components of the two-neuron reflex arc.^{38–40} A prolongation of the central delay of the total reflex latency is the most plausible explanation. The epidurally initiated afferent flow was routed through polysynaptic spinal pathways opened only at frequencies higher than 15 Hz and during induced burst-style activity. A concomitant action was the suppression of the monosynaptic component of the PRMRRs during the burst-style phases (Figure 5). We speculate that this is accomplished by recruitment and state-dependent modulations of spinal reflexes, which are nonfunctional during the ‘resting’ state of the

lumbar interneuronal circuits. Such modifications of reflex responses have also been shown during normal human locomotion.^{41,42} Furthermore, feline studies have shown that afferents may mediate their effects on motoneurons via different routes. This is true for large group-I afferents and for smaller high-threshold afferents from muscles, joints, and skin such as the ‘flexor reflex afferents’ (FRA) that activate common interneurons.^{43–47} Under conditions of intact connections between lumbar cord and brain stem structures, the flow of information into and through the central FRA pathways can be controlled by supraspinal centers.⁴⁸

PRMRRs and the lumbar locomotor pattern generator (LLPG)

Epidural stimulation with parameters that induced rhythmic flexion/extension movements of the lower limbs elicited afferent volleys via large diameter fibers within the posterior roots. The stimulated structures are a subset of sensory fibers that are involved in peripheral feedback. During locomotion, these sensory fibers transmit phasic input that enter the spinal cord via the posterior roots with spatially and temporally complex patterns. Epidural stimulation as described in the present study elicited a sustained, tonic input that was delivered simultaneously to several lumbar and upper sacral cord segments. Thus, the input was unlike physiological sensory information. We speculate, that – besides exerting facilitation of various motoneuron pools – the input acts as a common drive to spinal interneuronal networks located in the lumbar cord. While coming from periphery, the tonic input of particular frequencies is interpreted as a central command signal due to its code. The sustained stimuli organize lumbar spinal interneurons by temporarily combining them into functional units representing different levels of muscle synergies, parts of movements, or even more integrated motor behavior. The selection and activation of functional units is dependent on the frequency of tonic input. While at 5–15 Hz extension activity dominates the motor output, at 25–50 Hz the balance of neural activity is shifted to generate a rhythmic motor output. Our notion is that this can be achieved due to the flexibility of operation of spinal interneuronal networks and their multifunctional character⁴⁹ and due to the connectivity of the activated large diameter sensory neurons with these networks. Hultborn *et al*⁵⁰ have emphasized that different reflex pathways have direct access to, or may even be part of, the central pattern generator (CPG) for locomotion in the cat. Moreover, Burke *et al*⁵¹ stressed, that: ‘it has been known for some time that a variety of reflexes are modulated in amplitude and even reversed in sign during different phases of the stepping cycle, both in animals and man. Intracellular recordings from motoneurons during fictive locomotion have provided clear evidence that the locomotor CPG exerts powerful control of transmission through reflex pathways as assessed by phasic modulation of synaptic potentials.’

Can we conclude on the basis of the presented findings that the studied model of the human lumbar cord isolated from suprasegmental input by accidental injury has features of a CPG for locomotion? The hallmark for identification of a locomotor CPG within the spinal cord is the production of recognizable and reproducible patterns of rhythmic output in the absence of instructive external drive from higher levels of the central nervous system or from peripheral sensory feedback. So far we have demonstrated that by applying a sustained tonic input to the lumbar cord isolated from supraspinal influence, it is possible to activate and drive interneuronal networks and thereby to initiate stepping-like flexion/extension movements in the paralyzed lower limbs. To stabilize the induced motor activity, additional phasic sensory feedback from the lower limbs associated with the induced stepping-like movements was essential. This was shown in a study on the effects of temporarily reduced peripheral input when locomotor-like movements were evoked by SCS in paraplegics.⁵² While the parameters of epidural stimulation were maintained constant, it was observed that the reduced sensory feedback resulted in decreased amplitudes of the EMG activity and increased frequency of the lower limb movement. The phasic input had a timing function in the production of rhythmic movements and additionally augmented the activity of the rhythm-generating spinal circuits. We propose to consider the described capabilities of the human lumbar cord isolated from brain control and tested by repetitively induced PRMRs at 25–50 Hz as evidence for the existence of a LLPG.

There is a simultaneous progress in basic research on spinal reflex circuits,⁴⁷ organization of inputs to spinal interneuronal populations,⁵³ flexibility of operation of interneuronal circuits and final common interneuronal pathways,⁴⁹ and on central pattern generators for locomotion.⁵⁴ We should appreciate these recent findings of basic scientists regarding spinal interneurons when evaluating the present human neurophysiological study of the activation of spinal networks. Populations of spinal interneurons can be organized to act as functional units by tonic afferent input. Spinal interneurons are multifunctional and can be incorporated into different larger networks that exert defined facilitatory and inhibitory influences on various motoneuron pools. This flexibility was also shown in our study. By changing the repetition rate of tonic stimulation, a temporarily established pattern generator for stepping-like activity – the LLPG – was promptly converted to a pattern generator for lower limb extension.

The possibility of activating spinal networks involved in generating functional synergistic movements opens a new avenue with great potential for human neurophysiological studies of intrinsic spinal cord functional properties, and may contribute to the development of new methodologies, technologies, and clinical practice for restoration of movements in SCI people.

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