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Hindlimb Movement in the Cat Induced by Amplitude-Modulated Stimulation Using Extra-Spinal Electrodes

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Abstract

Hindlimb movement in the cat induced by electrical stimulation with an amplitude-modulated waveform of the dorsal surface of L5-S1 spinal cord or the L5-S1 dorsal/ventral roots was investigated before and after acute spinal cord transection at the T13-L1 level. Stimulation of the spinal cord or dorsal/ventral root at the same spinal segment induced similar movements including coordinated multi-joint flexion or extension. The induced movements changed from flexion to extension when the stimulation was moved from rostral (L5) to caudal (S1) spinal segments. Stimulation of a dorsal or ventral root on one side induced only ipsilateral hindlimb movement. However, stimulation on the dorsal surface of the spinal cord along the midline or across the spinal cord induced bilateral movements. The extension induced by stimulation of L7 dorsal root produced the largest ground reaction force that was strong enough to support body weight. Dorsal root stimulation induced a larger ground reaction force than ventral root stimulation and produced a more graded recruitment curve. Stepping at different speeds could be generated by combined stimulation of the rostral (L5) and the caudal (L6/L7) spinal segments with an appropriate timing between the different stimulation channels. Acute transection of the spinal cord did not change the responses indicating that the induced movements did not require the involvement of the supraspinal locomotor centers. The methods and the stimulation strategy developed in this study might be utilized to restore locomotor function after spinal cord injury.

Keywords

Spinal cord; Electrical stimulation; Locomotion; Hindlimb; Cat

Introduction

Direct muscle stimulation using intramuscular electrodes has been used to restore locomotor function after spinal cord injury (SCI) (Kobetic et al., 1997). However, several problems have been encountered including rapidly developing fatigue, electrode breakage, and the need to activate and control many muscles individually. In view of the problems with intramuscular stimulation, studies in recent years have been carried out using spinal cord stimulation with microelectrodes (Barthelemy et al., 2006, 2007; Guevremont et al., 2006; Lemay and Grill, 2004; Mushahwar et al., 2002; Saigal, 2004; Tai et al., 1999, 2000, 2003; Tresch and Bizzi, 1999). The initial motivation for using spinal cord microstimulation was based on: 1. Different groups of muscles are innervated by spinal motoneurons that are anatomically organized in different "motor pools" (Romanes, 1951; Vanderhorst and Holstege, 1997). Microelectrodes implanted in these "motor pools" can deliver focused electrical current to selectively activate

different groups of muscles (Mushahwar and Horch, 2000a); 2. During locomotion the electrodes and their leads in the spinal canal will experience less movement than the intramuscular electrodes, therefore less breakage (Tai et al., 1999, 2000); 3. Spinal cord microstimulation may activate a mixture of large and small muscle fibers resulting in a fatigue resistant movement (Mushahwar and Horch, 2000b).

However, our previous study (Tai et al., 2003) in cats showed that microstimulation of lumbosacral spinal cord using a single microelectrode induced coordinated, multi-joint hindlimb flexion or extension rather than individual muscle contraction or single joint movement. Other studies in cats (Barthelemy et al., 2006, 2007; Guevremont et al., 2006; Lemay and Grill, 2004; Mushahwar et al., 2002; Saigal, 2004), rats (Tresch and Bizzi, 1999), and frogs (Tresch et al., 1999) also showed that complex multi-joint movements could be activated by lumbosacral spinal cord microstimulation using a single electrode. Furthermore, bilateral hindlimb stepping in cats could be induced with only 2 microelectrodes implanted in the lumbosacral spinal cord (Mushahwar et al., 2002; Saigal et al., 2004; Tai et al., 2003). More recently, Barthelemy et al. (2006, 2007) reported that bilateral locomotion could be induced in chronic SCI cats by a single microelectrode implanted in the lumbosacral spinal cord. Activation by a single microelectrode of multiple segments of the spinal cord up to 17 mm rostral and caudal to the stimulation site has also been reported (Gaunt et al., 2006). These previous studies indicated that microstimulation via a single microelectrode did not selectively activate an individual “motor pool”, but rather activated a complex spinal neural network that could generate stereo-typed, multi-joint flexion or extension movements. The failure of microelectrodes implanted in the spinal cord to activate individual “motor pools” suggests that alternative, less invasive techniques might be equally effective in inducing motor activity. In this study we determined if electrical stimulation via extra-spinal electrodes could induce multi-joint flexion or extension movements similar to those elicited by stimulation using intra-spinal microelectrodes.

Extra-spinal stimulation using epidural electrodes has been performed in both animals and human subjects (Dimitrijevic et al., 1998; Gerasimenko et al., 2002, 2003, 2005; Ichiyama et al., 2005; Iwahara et al., 1992; Minassian et al., 2004; Jilge et al., 2004a,b; Pinter and Dimitrijevic 1999; Herman et al., 2002; Carhart et al., 2004). Bilateral hindlimb stepping movement was induced in both acute and chronic spinal animals on a running treadmill by an epidural electrode placed along the midline of the lumbosacral spinal cord (Gerasimenko et al., 2002, 2003, 2005; Ichiyama et al., 2005; Iwahara et al., 1992). Stepping-like movement was also induced in human subjects with complete SCI (Dimitrijevic et al., 1998; Gerasimenko et al., 2002; Minassian et al., 2004; Jilge et al., 2004a,b; Pinter and Dimitrijevic 1999). Recently epidural stimulation was reported to facilitate walking in individuals with an incomplete SCI (Herman et al., 2002; Carhart et al., 2004). Although stepping movements could be induced by epidural stimulation, the intensities of the stimuli used in these studies were constant which did not allow control or adjustment of each step’s amplitude and duration. The epidural stimulation had to be applied to a specific spinal segment at a certain intensity range in order to activate the intrinsic spinal locomotion network that determined the amplitude and duration of each step. At a higher stimulation intensity or in a different spinal segment, only tonic extension or flexion could be induced due to the constant intensity. Since the ability to control induced movements is very important in restoring locomotor function after SCI, an amplitude-modulated stimulation waveform was used in this study so that the tonic extension or flexion caused by the constant stimulation can be converted to a smooth movement with controllable amplitude and duration.

The long-term goal of our research is to develop a neural prosthetic device to restore locomotion after SCI. In this study we tested the hypothesis that extra-spinal electrodes can induce multi-joint flexion or extension movements that can be integrated into a stepping movement if the

amplitude-modulated stimulation is timed appropriately. First, the hindlimb movements were investigated by stimulating different spinal segments (L5-S1) in order to find the effective stimulation locations to induce a flexion or extension movement. Then, the hindlimb extension was further quantified by the induced ground reaction force. Finally, the amplitude-modulated stimulation was timed to alternatively induce flexion and extension in an attempt to generate a stepping movement. Some of the results have been published in abstracts (Tai et al., 2007; Wang et al., 2006).

Methods

Animal Preparation

All protocols involving the use of animals in this study were approved by the animal care and use committee of University of Pittsburgh School of Medicine. A total of 8 cats (2 male and 6 female, 2.8 kg to 4.3 kg) were studied under pentobarbital anesthesia (25 to 35 mg/kg, I.V. supplemented as necessary) after induction with isoflurane (2–3% in O₂). Systemic blood pressure was monitored throughout the experiment via a catheter inserted in the right carotid artery. A tracheotomy was performed and a tube was inserted to keep the airway patent. A catheter for I.V. infusion was introduced into the right ulnar vein. The spinal cord was exposed from L5 to S1 via a dorsal laminectomy. The spinal dura was opened and the dorsal and ventral roots were separated for electrical stimulation. The animal was mounted in a modified Narishige “Eccles” spinal cord frame in which the hip was supported by metal pins, and the spinous process at the laminectomy was secured with a clamp. The skin, cut mid-sagittally from L4 to S3, was tied along each margin to form a pool that was filled with warmed (35° to 37°C) mineral oil. A small laminectomy was also performed at T13-L1 level for acute spinal transection during the experiment, which was performed by first injecting small amount of lidocaine (0.2 ml) into the spinal cord, and then completely cutting the spinal cord. The 2–3 mm space between the cut ends of the spinal cord was filled with surgical cotton.

Experimental Setup

The left and right hindlimbs were video-taped from the left, right, and back sides using 3 digital cameras (JVC, GZ-MG50U). The hindlimb joints (hip, knee, and ankle) were marked by red cap pins for off-line analysis of the joint locations during the induced movement. A LED light was used to indicate the start and end of the stimulation. After the experiment, the digital videos were analyzed using video editing software (Pinnacle Studio 10). Stick figures of the hindlimb positions were drawn by connecting the red pins and paw on each video frame. The amplitude-modulated stimulation waveforms were generated by a computer with a digital-to-analog circuit board (National Instruments, AT-AO-10), which was programmed using LabView programming language (National Instruments). Linear stimulus isolators (World Precision Instruments, A395) were used to deliver constant current pulses to the animals. A platform force sensor (Arlyn Scales, Inc.) was used to record the ground reaction force that was amplified (Gould, Inc.), calibrated, and digitized via a computer using an analog-to-digital circuit board (National Instruments, PCI-6024E).

Stimulation Protocols

Each stimulus pulse was biphasic charge-balanced consisting of a short cathodic pulse (0.2 ms), an interpulse interval (0.2 ms), and followed by a low amplitude anodic pulse (0.6 ms). Biphasic charge-balanced stimulation will cause less tissue damage in chronic application (Agnew and McCreery, 1990). A train (40 Hz frequency, 2–12 second duration) of constant-current, biphasic pulses, the amplitude of which was modulated either by a 0.5 Hz sine waveform (see Fig. 1–3) or by a gradually ramped waveform (see Fig. 6–8) was used in this study. 40 Hz was used in order to induce a smooth, fused muscle contraction.

In the first group of experiments to identify the effective stimulation locations for inducing flexion or extension movement, the hindlimbs were allowed to move freely in the air. The stimulation was delivered to one of three sites: (1) the dorsal root; (2) the ventral root; (3) the dorsal surface of lumbosacral spinal cord. The stimulus pulse train modulated by an uniphasic sine wave (see Fig. 1) was used to stimulate each left dorsal or ventral root (L5-S1) using a hook electrode (stainless steel wires, 0.2 mm diameter, and 2 mm separation). The same stimulation waveform was also delivered by a bipolar electrode (stainless steel wires, 0.2 mm diameter, 1 mm length, and 2–4 mm separation) that was placed on the dorsal surface of each spinal segment (L5-S1) in the rostral-caudal direction along the midline or 1–2 mm lateral to the midline along the left or right dorsal root entry zone (see Fig. 2). A stimulus pulse train modulated by a biphasic sine wave (see Fig. 3) was also tested using bipolar stainless steel wire electrodes that were placed on the left and right dorsal root entry zone at the middle of each spinal segment. The biphasic sine wave stimulation was designed to alternatively activate the spinal cord on each side expecting to induce bilateral alternating movements. At each stimulation location, several supra-threshold stimulation intensities were tested in order to induce a moderate hindlimb movement.

The second group of experiments was designed to determine the ground reaction force induced by stimulation of the L7 or S1 segment that was shown to induce hindlimb extension. The left hindlimb was positioned to stand on the force platform with the paw fixed on the platform surface by tape. The right hindlimb was slightly elevated using a sling to lift the paw 1–2 cm above the force platform. To induce the ground reaction force the amplitude of the stimulus pulse train was gradually ramped up over a period of 1 sec, held constant for 2 sec at the maximal amplitude, and then slowly ramped down (1 sec). Stimulation was only applied to the left side at one of three sites: (1) the dorsal root; (2) the ventral root; (3) the spinal cord. Stimulation electrodes for spinal cord stimulation were placed on the dorsal surface of each spinal segment in the rostral-caudal direction along the left dorsal root entry zone (see Fig. 2). At each stimulation location, stimulation threshold (T) was determined and ground reaction forces were recorded at different stimulation intensities (1–4 T).

The third group of experiments was conducted to integrate the hindlimb flexion and extension movements induced at different stimulation locations into a stepping movement by appropriately timing the amplitude-modulated stimulation waveforms. Unilateral stepping was first investigated by allowing one hindlimb to move freely on the force platform with the other hindlimb slightly elevated above the platform. Then both hindlimbs were allowed to move freely on the force platform in order to induce a bilateral stepping. Since the hip of the animal was fixed on a frame, strong stimulation may extend the hindlimb into the air (see Fig. 1–3). To prevent this, a metal bar was positioned about 8–10 cm caudal to the resting location of the paw so that the paw could push against it when the hindlimb was fully extended.

In order to determine the supraspinal influence on the evoked responses and to avoid the effect of spinal shock due to acute spinalization, the experiments described above were repeated 2–3 hours following an acute spinal cord transection at the T13 - L1 segmental level.

Data Analysis

Multiple measurements of the ground reaction forces in the same animal under the same condition were averaged. In order to summarize the data from different animals, measurements of the ground reaction forces from the same animal were normalized to their maximal value (induced by stimulation of L7 dorsal root at 4T, see Fig. 4A). Then they were averaged across the animals and represented by mean $\pm$ SE. Paired t-test was used to detect significant differences ($P < 0.05$).

Results

Flexion or extension induced by stimulation of different spinal segment

Stimulation of a dorsal or ventral root on one side only induced ipsilateral hindlimb movements. The ipsilateral movements changed from flexion to extension (see Fig. 1 and Table 1A) as the stimulation of individual dorsal or ventral root was moved from a rostral spinal segment (L5) to a caudal spinal segment (S1). Table 1A summarizes the different hindlimb movements in 4 cats induced by stimulation of individual dorsal or ventral root between L5 and S1 on the left side. Stimulation of a dorsal or ventral root induced similar movement. Hindlimb flexion could be induced by stimulation of the L5 roots, but extension could be induced by stimulation of the L7/S1 roots. Stimulation of the L6 roots could induce either flexion or extension in different animals (see Table 1A). The induced movement shown in Table 1 was consistent with repeated stimulation at the same stimulation location in the same animal. Spinal transection did not change this segmental specificity. Only small adduction or abduction movement was induced. Adduction movements were mostly observed with the induced flexion, whereas abduction was more common with induced extension.

Stimulation on the dorsal surface of the spinal cord with electrodes placed along the length of the spinal cord induced both ipsilateral and bilateral hindlimb movements (see Fig. 2 and Table 1B). Table 1B summarizes the different hindlimb movements in 4 cats induced by stimulation of different segments of the spinal cord between L5 and S1. Stimulation on one side of the L5-L7 spinal cord (left or right) mainly induced ipsilateral hindlimb movement (square symbols in Table 1B), but bilateral movement (circle symbols in Table 1B) could be induced at the S1 spinal segment. The bilateral movement induced at S1 segment was probably due to the small size of the spinal segment (2–3 mm wide) and the large size of the stimulation electrodes (1 mm long), causing current spread into the contralateral side of the spinal cord. Bilateral hindlimb movements could also be induced when the stimulation location was in the middle of the spinal cord. The induced hindlimb movements changed from flexion to extension (from open symbols to dark symbols in Table 1B) when the stimulation (middle, left, or right) was moved from rostral (L5) to caudal (S1) spinal segment. This segmental change was maintained after spinal cord transection. A comparison between root stimulation and spinal cord stimulation (see Table 1A and B) shows that at the L6 segment the extension movement was induced by root stimulation more often than spinal cord stimulation.

In order to alternatively activate the spinal cord on each side, biphasic sine wave modulation with stimulation electrodes placed across each spinal segment was also tested (see Fig. 3). The induced bilateral hindlimb movements can be classified into 4 basic movement patterns (see Fig. 3A). In the first movement pattern, flexion of both hindlimbs during both the positive and negative phases of the modulation was induced. In the second movement pattern, extension of both hindlimbs during both modulation phases was induced. In the third movement pattern, flexion of one hindlimb but extension of the other in one phase with flexion of both hindlimbs in the following modulation phase were induced. The fourth movement pattern was similar to the third one, except that extension of both hindlimbs in one modulation phase was induced instead of flexion.

In addition to the synchronized movement of both hindlimbs as shown in Fig. 3A, alternating flexion movement of each hindlimb during different modulation phases was also observed in one cat (Fig. 3B). However, alternating extension of each hindlimb was never observed. In Fig. 3B bilateral air-stepping was induced by stimulation of the L6 spinal cord in a spinal intact cat. A total of 60 steps (30 steps for each hindlimb) were induced during continuous 60 second stimulation, but only 6 steps are shown in Fig. 3B. There was no observable fatigue during the 60 steps. Similar bilateral air-stepping was also induced in the same animal after acute spinal transection by stimulation of the L5 spinal cord (see Table 1C).

Although more complex bilateral hindlimb movement patterns were induced with the biphasic sine wave modulated stimulation (Fig. 3), the induced movement pattern still changed from flexion to extension when the stimulation was moved from a rostral segment (L5) to a caudal segment (S1) (see Table 1C). This rostral-to-caudal segmental change of movement was maintained after acute spinal cord transection. Compared to uniphasic modulation with stimulation electrodes placed along the midline of the spinal cord, biphasic modulation with electrodes placed across the spinal cord induced movements consisting of one hindlimb flexion and contralateral hindlimb extension at more stimulation locations (compare Table 1B to Table 1C).

Ground Reaction Force during Induced Hindlimb Extension

With the left hindlimb fixed on the force platform, the ground reaction force induced by electrical stimulation of the dorsal root, ventral root, or spinal cord of the L7/S1 segments on the left side changed greatly depending on the stimulation location and intensity. Stimulation of the L7 dorsal root generated the largest ground reaction force and produced a more graded recruitment curve than stimulation of the L7 ventral root (Fig. 4A-B and D-E). S1 dorsal root stimulation produced a much smaller force compared to L7 dorsal root stimulation (Fig. 4A and D). S1 ventral root stimulation only generated a very small force (Fig. 4B and E). The fact that stimulation of S1 segment generated comparable or larger extension movement than stimulation of L7 segment (see Fig. 1–2) indicated that S1 stimulation might generate an extension force directed more caudally than L7 stimulation. Spinal cord stimulation was only tested up to 2 T due to its high stimulation threshold (Fig. 5A), because our stimulation system could only provide up to 10 mA constant current. After acute spinal cord transection at T13-L1 level, the recruitment curves were largely unchanged. Paired t-test showed no significant difference ($P>0.05$) between the ground reaction forces generated before and after spinal cord transection.

The thresholds for stimulation of the L7 or S1 segment are plotted together in Fig. 5A since there was no significant difference ($P>0.05$) between the 2 data sets. The thresholds for ventral root stimulation were significantly ($P<0.05$) lower than both spinal cord and dorsal root stimulations. But the thresholds for dorsal root stimulation were not significantly ($P>0.05$) lower than spinal cord stimulation. Spinal cord transection at the T13-L1 level did not significantly ($P>0.05$) increase the stimulation threshold for either ventral root stimulation, dorsal root stimulation, or spinal cord stimulation.

The actual forces corresponding to the 100% normalized ground reaction forces induced by stimulation of the L7 dorsal root at an intensity of 4 T (see Fig. 4A) are shown in Fig. 5B for the 4 cats tested. Before spinal cord transection the ground reaction force in each animal was near or above 9.8 N (1 kg), i.e. strong enough to support a quarter of the body weight of a 4 kg cat. Spinal cord transection reduced the ground reaction forces in 3 cats, but they were still larger than a quarter of the cat body weight (except cat #6). Although a large reduction of ground reaction force was observed in cat #7, paired t-test showed that this change was not significant ($P>0.05$) among the 4 cats.

Integrating flexion and extension into stepping

The isolated flexion or extension movements as shown in Figs. 1–3 and Table 1 were converted into stepping movements by delivering several channels of amplitude-modulated stimulations to different locations in an appropriate time sequence. Fig. 6 shows how this conversion was achieved in the right hindlimb by stimulation of both L5 and L7 dorsal roots on the right side. The amplitude-modulated stimulation (see Fig. 6A) applied to the right L5 dorsal root induced flexion movements of increasing magnitudes as the stimulation intensity gradually increased. Stimulation intensity (3 mA) that moved the hindlimb from an extended position to a neutral

standing position was chosen to induce a hindlimb flexion during stepping (see Fig. 6B and C). Applying the amplitude-modulated stimulation (see Fig. 6A) to the right L7 dorsal root induced an extension of the hindlimb. The ground reaction force during each extension movement was also gradually increased as the stimulation intensity increased (see Fig. 6A). The stimulation intensity (2.3 mA) that generated about 10 N (about 1 kg) of ground reaction force was chosen to induce an extension during stepping (see Fig. 6B and C). By alternatively applying the amplitude-modulated stimulation as shown in Fig. 6A to the right L7 and L5 dorsal roots, an ipsilateral hindlimb stepping movement was generated with ground reaction force during each step strong enough to support the body weight (Fig. 6B). By shortening the duration of each amplitude-modulated stimulation waveform, the frequency of the induced right hindlimb stepping was increased from 4 sec/step to 2 sec/step (Fig. 6C). It is worth noting that the ground reaction force was below the baseline during flexion due to the fact that the hindlimb was lifted off the force platform (see Fig. 6B and C).

Stepping could still be induced after spinal cord transection. Fig. 7 shows that the right hindlimb stepping was induced by delivering 2 channels of amplitude-modulated stimulation to the L6 dorsal root (for extension) and the L5 spinal cord (for flexion) on the right side after the spinal cord was transected. The stepping speed in Fig. 7A was 4 sec/step, but was increased to 2 sec/step in Fig. 7B. Also note that the ground reaction force was below the baseline during flexion and it was strong enough (about 10 N) to support the body weight during extension.

Bilateral stepping could also be induced. Fig. 8 shows bilateral stepping induced by delivering 4 channels of amplitude-modulated stimulation to the L7 and L5 dorsal roots on both sides. Note that the left and right stimulations were timed to induce an extension on one side while inducing a flexion on the contralateral side. The ground reaction force was never reduced to zero indicating that there was always one paw touching the force platform during the stepping.

Fig. 9 summarizes the distances that the foot traveled during the induced stepping movement in the 5 cats tested. Except for cat # 6 where the induced flexion movement failed to bring the foot across the hip joint axis, the foot was able to travel a relatively large distance (about 5–10 cm) in the other 4 cats.

Discussion

This study showed that coordinated, multi-joint flexion or extension movements could be induced by amplitude-modulated stimulation of the lumbosacral spinal cord or dorsal/ventral roots using extra-spinal electrodes (Fig. 1–3 and Table 1). The induced hindlimb movements changed from flexion to extension when the stimulation was moved from a rostral segment (L5) to a more caudal segment (L7/S1). Stimulation of the L7 dorsal root induced the largest ground reaction force with a graded recruitment curve (Fig. 4), which was strong enough to support the body weight (Fig. 5). Stepping at different speeds could be generated by combined stimulation of the rostral (L5) and the caudal (L6-L7) spinal segments with an appropriate timing between the different stimulation channels (Fig. 6–9). Acute transection of the spinal cord at T13-L1 level did not change the results (Fig. 1–5 and Table 1) indicating that the induced movements do not require the involvement of the supraspinal locomotor centers.

Novelty and limitations of this study

The novelty of this study lies in that it combined the extra-spinal electrodes with the use of amplitude modulated stimulation waveforms. Due to the problems encountered in intramuscular stimulation (i.e. fatigue, electrode breakage, and requiring many stimulation electrodes etc.), recent studies to design neuroprosthesis for locomotion have been carried out using intra-spinal microstimulation. Although the results similar to those reported in this study were also obtained using intra-spinal microelectrodes in our previous studies (Tai et al.,

1999, 2000, 2003) and others (Barthelemy et al., 2006, 2007; Guevremont et al., 2006; Lemay and Grill, 2004; Mushahwar et al., 2002; Saigal, 2004; Tresch and Bizzi, 1999), intra-spinal microstimulation is invasive to the spinal cord and may not be applicable clinically in the foreseeable future. However, the extra-spinal electrodes including epidural spinal electrodes (Dimitrijevic et al., 1998; Gerasimenko et al., 2002; Minassian et al., 2004; Jilge et al., 2004a,b; Pinter and Dimitrijevic 1999; Herman et al., 2002; Carhart et al., 2004) and the cuff electrodes on the spinal roots (Brindley and Rushton, 1990; Donaldson et al., 1997a,b; Rushton et al., 1997) are currently being used in clinical practice. Therefore, the stimulation strategy and techniques developed in our study could be easily tested clinically using extra-spinal electrodes similar to those that have already been implanted in patients. Our study is different from other studies that also used extra-spinal electrodes (Dimitrijevic et al., 1998; Gerasimenko et al., 2002, 2003, 2005; Ichiyama et al., 2005; Iwahara et al., 1992; Minassian et al., 2004; Jilge et al., 2004a,b; Pinter and Dimitrijevic 1999; Herman et al., 2002; Carhart et al., 2004) because we used amplitude modulated stimulation waveforms rather than a stimulation of constant intensity. These stimulation waveforms enable us to control not only movement amplitude (Fig. 4 and Fig. 6A) but also the speed of the stepping movement (Figs. 6–7). Epidural spinal cord stimulation employing a constant stimulation intensity does not allow control or adjustment of stepping amplitude and duration, and diminishes its usefulness in applications to restore locomotor function after SCI. Our study demonstrates for the first time the possibility in animal experiments that the extra-spinal electrodes can be used to restore locomotion after SCI if stimulation waveforms are amplitude modulated.

It is important to note that this study used pentobarbital anesthesia, which may have an influence on the reflex motor activity. A previous study (Mushahwar et al., 2004) using intra-spinal microstimulation has shown that the induced movements could change significantly in cats that were anesthetized, decerebrated, and then spinalized. These changes could have occurred with an extra-spinal stimulation as well. The ability to induce locomotion in decerebrated cats without anesthesia using the approach presented in this study needs to be tested. Our study in anesthetized animals is aimed at developing a neuroprosthesis to restore locomotion for SCI people. The neural control of locomotion in humans is significantly different from that in cats. The movements induced in human SCI subjects might be completely different from the animals regardless of the experimental conditions. However, the methods to develop the stimulation strategy presented in this paper i.e. identifying the effective stimulation locations to induce an isolated flexion or extension movement and generate ground reaction force strong enough to support the body weight, and then integrating these isolated movements into a stepping movement using amplitude modulated stimulation waveforms will be surely applicable to human studies.

Segmental responses and activation of multiple spinal segments

The induced hindlimb movements changed from flexion to extension when the stimulation was moved from L5/L6 to L7/S1 (Fig. 1–3 and Table 1). Our previous study (Tai et al., 2003) using microelectrodes implanted in the cat spinal cord also showed that coordinated, multi-joint flexion could be induced by microstimulation in the ventral areas of L5/L6 spinal cord, but extension could be induced in L7/S1 ventral areas. Other studies using cats (Barthelemy et al., 2006; Mushahwar et al., 2004; Aoyagi et al., 2004) also showed that the hindlimb movements induced by spinal cord microstimulation or stimulation of dorsal/ventral roots changed from forward/flexion movement to backward/extension movement when the stimulation location was changed from rostral (L5/L6) to caudal (L7/S1) segments. Neuroanatomical studies in cats (Romanes, 1951; Vanderhost and Holstege, 1997) revealed that the motoneurons innervating hip flexors (iliopsoas and sartorius) are mainly located in the L5–L6 segments, while the motoneurons innervating hip extensors (hamstrings, gluteus medius and maximus, caudofemoralis, abductor cruris caudalis, and obturatorius internus) are located

in the L7-S1 segments. The rostral-caudal distribution of the hip motoneurons agrees well with the electrically induced hindlimb flexion or extension movements at different spinal segments (Fig. 1–3 and Table 1). However, knee extensor motoneurons (quadriceps) are also located in the L5-L6 segments together with the hip flexor motoneurons. Electrically stimulating the L5-L6 segments induced coordinated multi-joint flexion rather than hip flexion with a knee extension. Meanwhile, knee flexor motoneurons (hamstrings, gastrocnemius medialis and lateralis) are located in the L7-S1 segments together with the hip extensor motoneurons. Electrically stimulating the L7-S1 segments induced coordinated multi-joint extension rather than hip extension with a knee flexion. The discrepancy between the induced knee joint movements and the distribution of the knee flexor and extensor motoneurons indicates that motoneuron activation was not limited to a single spinal segment although the stimulation was applied only to a single spinal segment. The activated motoneurons must have involved flexor motoneurons (or extensor motoneurons) in several spinal segments controlling multiple joints.

The spread of neuronal activation induced by electrical stimulation of a single spinal segment into other segments of lumbosacral spinal cord may occur directly by activation of the intraspinal pathways or reflexively by the sensory feedback once the movement was induced. Stimulation of a single dorsal root could directly activate multiple spinal segments since afferent fibers in a single dorsal root have an extensive distribution to multiple spinal cord segments (Culberson and Brown, 1984). Stimulation of the dorsal surface of spinal cord could also directly activate the ascending or descending nerve fibers running in the dorsal and/or dorsolateral columns (Coburn and Sin, 1985; Gerasimenko et al., 2002) that have synaptic connections with the interneurons in several spinal segments (Bras et al, 1989). These interneurons form the spinal neural network (or CPG) for locomotion that could activate hindlimb motoneurons in the ventral horn along the length of the lumbosacral spinal cord in a synergistic manner to induce coordinated, multi-joint movements. A recent study (Gaunt et al., 2006) has shown that microstimulation via a single microelectrode implanted in the lumbosacral spinal cord could antidromically activate multisegmental sensory afferents.

Once the hindlimb movement is induced, it could further reflexively activate the motoneurons in multiple spinal segments via the sensory feedback through the dorsal roots of multiple spinal segments. This view is suggested by the finding in this study that ventral root stimulation activated not only the directly innervated muscles, but also many other synergistic muscles through reflex activation via the dorsal roots once the movement is induced. In the same segment, ventral root stimulation generated movement patterns (flexion or extension) similar to those induced by dorsal root stimulation suggesting that both stimulations might elicit a similar multisegmental activation of the spinal motoneurons directly and reflexively. The fact that the dorsal or ventral root stimulation induced movements similar to those induced by spinal cord stimulation (Fig. 1–3 and Table 1) further indicated that multisegmental reflex activation via the afferent fibers in the dorsal roots played a more dominant role than the direct activation by the electrical stimulation.

Stimulation of the spinal roots to induce locomotion

The afferent axons in the dorsal roots might be the preferred target for electrical stimulation to restore locomotion after SCI. Although stimulation of the dorsal/ventral root or spinal cord at the same spinal segment induced similar movements (Fig. 1–3 and Table 1), dorsal root stimulation generated a larger ground reaction force than ventral root stimulation and also produced a more graded recruitment curve (Fig. 4). Compared to spinal cord stimulation, dorsal root stimulation required a lower stimulation intensity (Fig. 5A). A recent study (Barthelemy et al., 2007) has shown that stimulation of afferent axons in the dorsal roots could induce locomotor responses similar to those evoked by intra-spinal microstimulation. The results in our study further showed that direct stimulation of the dorsal root afferents using amplitude-

modulated stimulation induced stepping movements in which the stepping speed and the amplitudes of extension/flexion movements were controlled by the stimulation (Fig. 6–8).

In acute spinal (T12) cats, treated with DOPA, curarized or with all lumbosacral dorsal roots transected (Sjostrom and Zangger, 1976; Grillner and Zangger, 1979), constant stimulation of the central ends of the transected dorsal roots (L5-S1) induced “fictive locomotion” that involved alternative activation of both flexors and extensors. Stimulation of the dorsal or ventral roots to induce hindlimb movement was also studied recently in cats (Aoyagi et al., 2004) with the dorsal and ventral roots sequentially cut during the experiments. Transection of the dorsal roots reduced or eliminated the spinal reflex activation during the induced movements making it difficult to compare the results with our study. However, both extension and flexion of the hindlimb could still be induced by dorsal or ventral root stimulation even with the dorsal roots transected sequentially. Stimulation of the lumbar anterior roots to induce standing or stepping in humans after SCI was also reported (Donaldson et al., 1997a,b; Rushton et al., 1997). In this study we further showed that a weight-bearing stepping movement could be generated by stimulation of dorsal roots at different spinal segments using amplitude-modulated stimulation (Fig. 6–8).

Epidural stimulation of the spinal cord to induce locomotion

Epidural stimulation of the spinal cord has also been conducted in cats (Iwahara et al., 1992; Gerasimenko et al., 2002, 2003, 2005), rats (Ichiyama et al., 2005), and humans (Carhart et al., 2004; Dimitrijevic et al., 1998; Gerasimenko et al., 2002; Herman et al., 2002; Minassian et al., 2004; Jilge et al., 2004a,b; Pinter and Dimitrijevic 1999) in an attempt to restore locomotor function after SCI. The epidural stimulation is aimed at activating the spinal central pattern generator (CPG) for locomotion. Therefore, only stimulation of a constant intensity was used in these studies to mimic the constant input from the supraspinal locomotor centers. The constant stimulation resulted in either a tonic flexion or extension when it was applied at most of the spinal segments, requiring the epidural stimulation to be applied to a specific spinal segment in order to induce locomotion. Meanwhile, the stimulation intensity had to be within a certain range to activate the CPG and avoid the tonic activation. In order to induce locomotion, a running treadmill was used to move the hindlimb backward during each step (Ichiyama et al., 2005; Iwahara et al., 1992; Gerasimenko et al., 2002, 2003, 2005). The speed of the stepping induced by epidural spinal cord stimulation was influenced by the speed of the running treadmill rather than the stimulation. The ground reaction force generated by epidural stimulation during stepping on a treadmill was not strong enough to support the body weight (Ichiyama et al., 2005; Iwahara et al., 1992). In our study, an amplitude-modulated stimulation waveform was used which allowed the duration and magnitude of the induced flexion or extension movement to be adjusted by the stimulation (see Fig. 4 and Fig. 6A). A running treadmill was not required to move the hindlimb backward. Instead, the stimulation induced the hindlimb to move backward during the stepping generating ground reaction forces strong enough to support the body weight (Fig. 4, Fig. 5B and Fig. 6–8). The stepping speed was controlled by the amplitude-modulated stimulation rather than a running treadmill (Fig. 6–7).

Laterality of the induced movement

Stimulation of dorsal or ventral roots on one side only induced ipsilateral hindlimb movements (Fig. 1 and Table 1A); whereas bilateral hindlimb movements were induced when stimulation electrodes were placed along the midline of the spinal cord or across the spinal cord (Fig. 2–3, and Table 1B and C). A previous study (Grillner and Zangger, 1979) on the spinal generation of locomotion in cats suggested that there is at least one spinal neural network for each hindlimb. This ipsilateral organization allowed an independent control of each hindlimb during the induced stepping (Fig. 6–8). It is worth noting that the ground reaction forces as shown in Fig. 8 were unbalanced between the left and right sides during the stepping. However, this

unbalance could be adjusted by either increasing the intensity of left L7 dorsal root stimulation or decreasing the intensity of right L7 dorsal root stimulation due to the ipsilateral organization of the dorsal root induced responses (see Fig. 1, Fig. 6A, and Table 1A).

Both uniphasic and biphasic modulation (see Fig. 2–3) were evaluated in this study to stimulate the spinal cord. The biphasic modulation was designed to alternatively activate the spinal cord on each side (Fig. 3). Although it could induce movements bilaterally (Fig. 3A), an alternating flexion movement between the left and right hindlimbs (Fig. 3B) was only observed in one cat (see Table 1C). Alternating extension movements were never observed. This result indicates that using the biphasic modulation to alternatively activate the spinal cord on each side might not be useful for inducing stepping movements. However, uniphasically modulated stimulation applied along the midline of the L7-S1 spinal cord (see Fig. 2) might be effective in inducing a bilateral standing posture.

Potential clinical application

Only an open-loop control method using a pre-determined stimulation waveform was employed in this study to test our hypothesis that the extra-spinal electrodes can induce flexion, extension, or stepping movements just like those induced by intra-spinal microelectrodes. However closed-loop feedback control methods will be surely needed in clinical applications in order to improve the accuracy and stability of the induced locomotion. Extra-spinal electrodes which are less invasive than the intra-spinal microelectrodes have been used clinically to electrically stimulate the spinal roots (Brindley and Rushton, 1990; Donaldson et al., 1997a,b; Rushton et al., 1997) or the spinal cord (Herman et al., 2002; Carhart et al., 2004), which makes it easier to translate the concepts and techniques developed in this study into clinical application. The hindlimb flexion, extension, or stepping movements induced in this study were largely un-changed after acute spinal cord transection further indicating that supraspinal locomotor centers are not required. Therefore, the methods and stimulation strategy used in this study might be useful in restoring locomotor function in people with SCI.

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Fig. 1.

Ipsilateral left hindlimb movements induced by stimulation of individual left dorsal or ventral roots before and after spinal cord transection. Maximal stimulation intensity: 1 mA. Stimulation waveform represents the relative current levels. All stick drawings are from left camera view. The thin stick drawings show the resting position of the left hindlimb. The thick stick drawings show the maximal hindlimb movement during the stimulation.

Fig. 2.

Bilateral hindlimb movements induced by stimulation applied on the dorsal surface of different spinal segment (L5-S1) before (A) and after (B) spinal cord transection. Maximal stimulation intensities: 1–6 mA. Stimulation waveform represents the relative current levels. The thin stick drawings show the resting position of left or right hindlimb. The thick stick drawings show the maximal hindlimb movement during the stimulation. When no movement is induced, the hindlimb position is represented by its resting position only. In each column the left and right hindlimbs are shown as they are viewed from the left or right camera.

Fig. 3.

A. Four basic patterns of bilateral hindlimb movements induced by biphasic sine modulation with electrodes across each spinal segment. The thin stick drawings show the resting position of each hindlimb. The thick stick drawings show the maximal hindlimb movement during positive or negative phase of stimulation. B. Bilateral air-stepping induced by stimulation of the L6 spinal cord in a spinal intact cat. Maximum stimulus amplitude was 1 mA. Total of 60 steps (30 steps for each hindlimb) were induced during continuous 60 second stimulation. Only 6 steps are shown in this figure. Stimulation waveform represents the relative current levels.

Fig. 4.

Normalized ground reaction forces of the left hindlimb induced by stimulation of the left dorsal root (A and D), the left ventral root (B and E), or the left spinal cord (C and F) at L7 (○) or S1 (●) segment before (A, B, and C) and after (D, E, and F) spinal cord transection at stimulation intensity of 1–4 T. N = 4 (Cat #: 5–8).

Fig. 5.

A. Average thresholds for stimulation of ventral root, dorsal root, or spinal cord. * indicates statistical significance ($P < 0.05$). $N = 10$. Data were from 5 cats and the thresholds for stimulation of L7 or S1 segment were plotted together. B. Ground reaction forces induced by stimulation of the L7 dorsal root at intensity of 4 T.

Fig. 6.

Integrating flexion and extension movements into stepping. A. Ipsilateral flexion or extension of right hindlimb induced by stimulation of right L5 or L7 dorsal root separately. B. Ipsilateral stepping (4 sec/step) of right hindlimb induced by a combined stimulation of right L5 and L7 dorsal roots. C. Ipsilateral stepping of right hindlimb at a faster speed (2 sec/step). Amplitude-modulated stimulation pulses is shown in A, but only the modulation waveforms are shown in B and C. The modulation waveforms only indicate the timing. The actual stimulation intensity was marked separately. Stimulation sites and the camera view of right hindlimb are also shown in A. The measurement of each joint angle was indicated in A on the camera view. Data are from a spinal intact cat.

Fig. 7.

Ipsilateral stepping of right hindlimb induced by a combined stimulation of right L5 spinal cord and L6 dorsal root after spinal cord transection. A. At a stepping speed of 4 sec/step. B. At a stepping speed of 2 sec/step. Only modulation waveforms are shown for stimulation. Stimulation sites for both A and B are shown in A. The modulation waveforms only indicate the timing. The actual stimulation intensity was marked separately. The measurement of each joint angle was indicated in Fig. 6A on the camera view.

Fig. 8.

Bilateral stepping induced by stimulation of L5 and L7 dorsal roots on both sides. Only modulation waveforms are shown for stimulation. The modulation waveforms only indicate the timing. The actual stimulation intensity was marked separately. The ground reaction force generated by right or left hindlimb is marked by a letter “R” or “L”. Data are from a spinal intact cat.

Fig. 9.

Average distances the foot traveled during the induced stepping movements in the 5 cats tested.

- - maximal foot location during extension relative to the axis passing through the hip joint.
- - maximal foot location during flexion relative to the hip axis.

Table 1

Hindlimb movement patterns induced by stimulation of individual spinal roots or different segment of the spinal cord. Data are from 4 animals (Cat #: 1–4). Each column represents data from one animal.

A. Dorsal or Ventral Root Stimulation (see Fig.1)

Spinal Segment	Spinal Intact Cats		Spinal Transected Cats	
	Dorsal Root	Ventral Root	Dorsal Root	Ventral Root
L5	□ □ □ □	- □ - □	□ □ □ □	- □ - □
L6	□ □ □ ■	■ □ ■ ■	■ □ ■ ■	■ □ ■ ■
L7	■ ■ ■ ■	■ ■ ■ ■	■ ■ ■ ■	■ ■ ■ ■
S1	- ■ ■ ■	- ■ ■ ■	- ■ ■ ■	- ■ ■ ■

B. Spinal Stimulation along the Spinal Cord (see Fig.2)

Spinal Segment	Spinal Intact Cats			Spinal Transected Cats		
	Left	Middle	Right	Left	Middle	Right
L5	x □ x □	□ ○ □ ○	□ □ □ □	□ □ □ □	□ x x ○	□ □ □ □
L6	□ □ □ □	○ ○ x ●	□ □ x □	□ □ □ □	x x □ ○	□ □ □ □
L7	■ □ ■ ■	● ○ x ●	■ □ ■ ■	■ □ □ ■	■ □ ○ ●	■ □ ■ ■
S1	● ● ■ ■	● ⊗ ● ●	● □ ● ■	■ ● ■ ■	● ■ ● ●	■ □ ■ ■

C. Spinal Stimulation across the Spinal Cord (see Fig.3)

Spinal Segment	Spinal Intact Cats	Spinal Transected Cats
L5	□ ○ x ⊗	△ □ ○ ○
L6	△ ○ □ ⊗	○ ○ ⊗ ⊗
L7	⊗ ⊗ x ●	● ⊗ ⊗ ⊗
S1	● ⊗ ● ●	● ● ● ●

□ Ipsilateral hindlimb flexion

• Ipsilateral hindlimb extension

○ Bilateral hindlimb flexion.

△ Bilateral hindlimb extension.

⊗ One hindlimb flexion and the contralateral extension as shown in Fig 3A for patterns #3 and #4.

▲ Bilateral air stepping as shown in Fig. 3B.

x No movement.

-Not tested