The Hong Kong Polytechnic University

Department of Rehabilitation Sciences

Activities of Cortical Motor Neurons Trigger Electrical Stimulation of Lower Motor Neurons in the Spinal Cord

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A thesis submitted in partial fulfillment of the requirements of the Degree of Doctor of Philosophy.

March 2011

CERTIFICATE OF ORIGINALITY

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it reproduces no material previously published or written, nor material that has been accepted for the award of any other degree or diploma, except where due acknowledgement has been made in the text.

_____ (Signed)

LI Yan (Name of Student)

To my parents, husband and daughter, all my best blessings.

ABSTRACT

Restoration of hindlimb locomotion after spinal cord injury (SCI) remains a challenging problem even though functional electrical stimulation (FES) and neuromotor prostheses (NMP) have been investigated in previous studies. Since complete lesions in the lumbar spinal cord cause caudal section below the lesion cannot receive descending signals from the brain, the volitional control of hindlimb is damaged. In this study, to restore the voluntary movement of paralyzed hindlimb, a circuit was designed that can bypass a lesion to the spinal cord, linking upper motor neurons (UMNs) and lower motor neurons (LMNs) in guinea pigs. This circuit, named as "Motolink", which contains an amplifier and a stimulator. An amplifier acquired signals from the primary motor cortex (M1) via multi-electrodes array, which triggered a programmed microprocessor to generate pulse trains that directly activated LMNs, hence produced hindlimb movements.

The study was divided into two experiments. The first has been performed acutely, in which we used signals from visual cortex (VC), auditory cortex (AC) and medial geniculate body (MGB) respectively to induce hindlimb movement on anaesthetized rats with incomplete spinal cord injury. The second is chronic research, during which signals from hindlimb region of the primary motor cortex (M1HL) were acquired to induce contralateral lower limb action on guinea pigs with complete SCI, when animal slowly running on treadmill by their forelimbs with partial body support, or when animal moving freely.

The animal's spinal cord at the level of T12 was completely transected to serve as a SCI model. The recording electrode array was implanted into special area in the right

side of the brain. The stimulation electrode array was implanted into the anterior corner of the left L2-L3 segments in the spinal cord. During recording, Motolink was anchored on the skull to execute connection between cerebrum and spinal cord. Amplified cortical activities may be sent to remote computer for analyses simultaneously. One dimension of kinematics was described by Vicon system. The electromyography (EMG) activity of forelimb was recorded by another amplifier in chronic experiment part. Locations of recording and stimulating electrodes were examined by histology analyses.

In the anesthetized rat, our findings showed that neural response of VC, AC or MGB to stimuli of light, sound or electrical current can be exactly acquired by the circuit as the frequency of stimuli changed. Twitching of lower limb was observed every time when neural signals were acquired to activate LMNs. In chronic experiments, the artificial circuit chronically rerouted neuronal signals around the spinal injury, enabling twitching-like movements of the paraplegic, stimulated hindlimb as animals walked at different speeds on a treadmill by forelimbs. As shown in the corresponding videos, mostly each step of forelimb is followed by one movement of left hindlimb, but sometimes followed by none or two. The time lag between the forelimb and hindlimb was shortened significantly from 153+/-42 ms to 92+/-23 ms ($p<0.01$) when the speed of the treadmill was increased from 5.6 cm/s to 11.1 cm/s.

The UMNs were reconnected with LMNs by circuit in transected spinal cord, thus produced hindlimb movement, which electronically bypasses lesion in the spinal

cord. Our finding shows the possibility of a novel therapy for functional locomotion recovery of hind limbs in patients with spinal cord injury.

Key words: functional electrical stimulation; lower motor neuron; primary motor cortex; spinal cord injury; upper motor neuron.

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LIST OF ABBREVIATIONS

Abbreviation	Full name
A/D	Analog to digital
A	Anterior
AC	Auditory cortex
ALS	Amyotrophic lateral sclerosis
ANOVA	Analysis of variance
AP	Action potential
BCIs	Brain computer interfaces
C	Caudal
CPG	Central pattern generator
cm/s	centimeter per second
CNS	Central nerve system
CST	Corticospinal tract
El	Eyelid
ECoG	Electrocorticographic
EEG	Electroencephalography
EMG	Electromyography
EMS	Epidural microstimulation
ESCS	Epidural spinal cord stimulation
FBP	Furry buccal pad
FDA	Food and Drug Administration
FES	Functional electrical stimulation

FL	Forelimb
FM	Frequency modulation
FSC	Fetal spinal cord
g	gram
HL	Hindlimb
Hz	hertz
ICMS	Intracortical microstimulation
ISI	Inter-stimulus interval
ISMS	Intraspinal microstimulation
L2	The second level of lumbar spine
L4	The forth level of lumbar spine
LFP	Local field potential
LHL	Left hindlimb
LJ	Lower jaw
LMN	Lower motor neuron
MΩ	mega ohms
M1	Primary motor cortex
M1FL	Forelimb area of primary motor cortex
M1HL	Hindlimb area of primary motor cortex
MCU	Micro controller unit
MGB	Medial geniculate body
mm	millimeter
N	Nose
NIH	National Institutes of Health

NMP	Neuromotor prosthesis
NP	Neuroprostheses
NPs	Neural prostheses
NSPCs	Neural stem/progenitor cells
P	Posterior
PO	Perioral
R	Rostral
RF	Radio frequency
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
SC	Spinal cord
SCI	Spinal cord injury
SD	Standard deviation
T12	The twelfth level of thoracic spine
TDT	Tucker-Davis Technologies
TR	Trunk
UMN	Upper motor neuron
UZ	Unresponsive zone
V	Vibrissae
VC	Visual cortex
Ver.	Version

CHAPTER 1 INTRODUCTION

1.1 Background of This Study

Spinal cord injury is epidemic disease due to damage in interneuron, motoneuron and fibers of the spinal cord, which results in paralysis, physical disability and loss of social independent. Many causes may lead to spinal cord injury. Presently traumatic reason is the main cause for SCI. According to the data from official website, annual incidence of SCI is 22 per million of population internationally. The total number of people surviving this disease is over 130,000 people each year. In Hong Kong, there are a few hundreds of SCI patients need to be treated every year (Chan, Lee et al. 2000; Hu, Mak et al. 2008).

Patients with SCI usually suffer from paralysis in forelimbs or hindlimbs, sensory loss, continuous pain, dysuria, pressure ulcers and other infections (Rossignol, Schwab et al. 2007). Disease of spinal cord injury makes the patients loss of independence and brings burdens to their families and community economically and spiritually. Therefore, it is important to develop effective treatments for people with SCI to restore normal function and improve their quality of life.

However, recovery from spinal cord injury remains an insuperable medical challenge in current world. The challenge lies in not only technological difficulties but also the selection from diverse therapeutic strategies. Stem cell transplantation (McDonald, Liu et al. 1999; Garbossa, Fontanella et al. 2006), neuroprostheses (Schwartz 2004; Hochberg, Serruya et al. 2006; Velliste, Perel et al. 2008) and peripheral functional electrical stimulation (Ferguson, Polando et al. 1999; Fromm,

Rupp et al. 2001; Smith, Mulcahey et al. 2001; Moritz, Perlmutter et al. 2008) are all potential treatments for spinal cord injury. But each treatment has its advantages and disadvantages respectively. Combination of various treatment s in the therapy of spinal cord injury is better than application of only one. In addition, to explore a new high efficient and more accurate strategy for SCI patients is required.

1.2 Design and Result of This Study

Complete spinal cord injury causes the spinal cord below the level of lesion not to receive command signals from the descending never system. In spinal preparations the spinal cord is transected at the lower thoracic level which separates the spinal segments that control the hindlimb muscles from supraspinal center. However, the supraspinal structures in the brain and the spinal cord above and below the lesion remain relatively intact. In this study we proposed to bridge the broken neural pathway from the brain to the spinal cord by artificial bypass for restoration the locomotion of paralyzed extremity, as Figure 1.1 shows. Our aim is to find a novel therapeutic measure for the rehabilitation of patients with spinal cord injury.

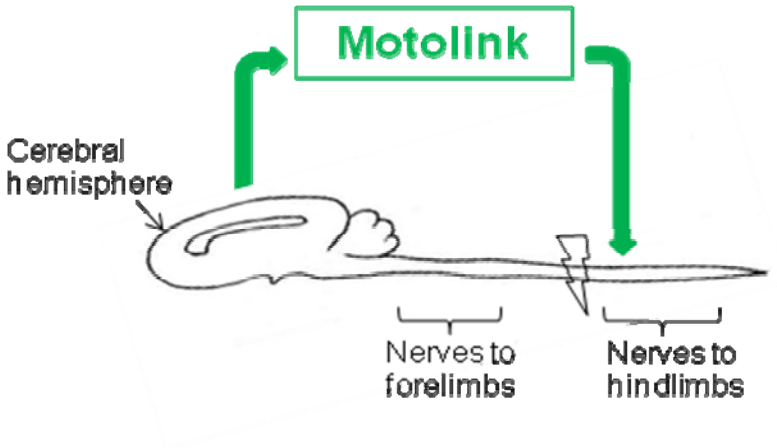

Figure 1.1 Motolink bridges the broken neural pathway from the brain to the spinal cord.

An electronic circuitry, given the name of "Motolink", was developed to achieve reconnection. It can produce intraspinal stimulation triggered by extracellular neural

activity from the motor cortex. The schematic of signal flow in Motolink applied on animals is showed in Figure 1.2. When Motolink works, neural activities from the brain of rats can be acquired and amplified by the amplifier. After electronic converting to a train of stimulation pulses by the stimulator, the stimulation is electrically transmitted to the contralateral ventral horn of the spinal cord below the lesion site. The stimulation can then activate motoneurons and thus elicited corresponding muscles response to produce locomotion such as flexion or extension in the hindlimb. In this study, signals acquired from one channel in the cortex are bridged to stimulate a small group of motoneurons of one channel in the spinal cord.

The experiment was divided into two sessions, acute experiments at first and chronic experiments on the following. In acute experiments as testing system, neural signals were selected from medial geniculate body (MGB), visual cortex (VC) and auditory cortex (AC) that were easily acquired in anaesthetized status when motor signals are not. It explored the effect of Motolink in connecting the brain and the spinal cord even though these signals are not movement volition. In chronic experiments, neural activities were acquired from hindlimb area of the primary motor cortex for voluntary control of hindlimb movement in awake animals. The main differences between acute and chronic experiments are the signal sources from the brain and their purpose.

Our findings show successful reconnection of the brain and the spinal cord below the injury by using self-developed Motolink in anaesthetized rats and conscious guinea pigs. In acute experiments, we found that neural response of MGB, VC or AC to stimuli of light or sound could be exactly acquired by Motolink as the frequency of stimuli and inter stimulus interval (ISI) changed. And twitching of lower limb was

observed when spinal cord motoneurons were activated by respective neural response. Our findings showed neurons in the medial geniculate body, auditory cortex and visual cortex were connected with the spinal motoneurons below the lesion in anaesthetized rats.

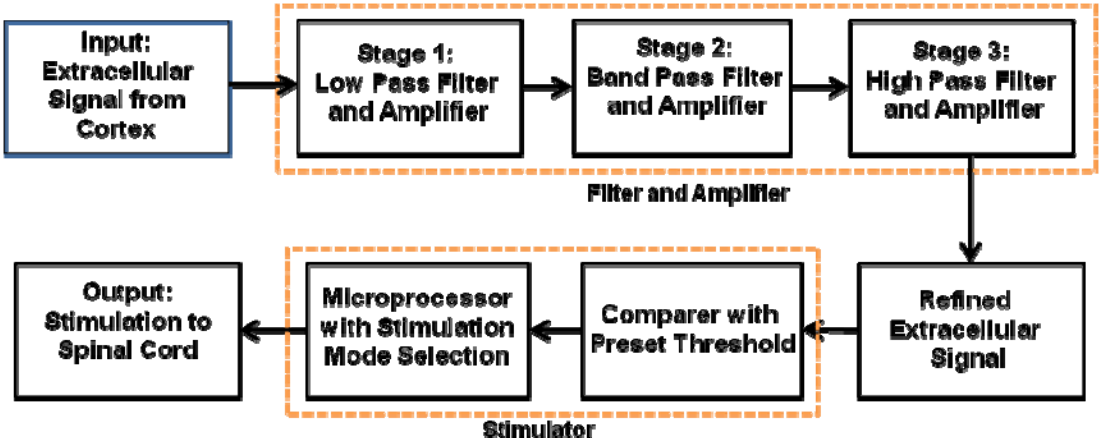

Figure 1.2 Schematics program of Motolink. Input cortical signal after three stages of filter and amplification is compared with preset threshold and upper threshold signal will be transformed into pulse stimulation, which is the output to spinal cord. The orange dashed outline contains the components of Motolink, which has an amplifier and a stimulator. A connector has the input of neuronal signal and output of stimulation signal which to be held on the head of guinea pig.

In chronic experiments, we clearly showed that twitching of left hindlimb was subsequent to each step of left forelimb as guinea pig walking on the treadmill. Time lag between electromyography (EMG) activity of forelimb and hindlimb was statistically decreased as the speed of treadmill increased. Cross-correlation shows high correlation between the EMG activity of forelimb and the spikes of hindlimb region of the primary motor cortex. As shown in supplementary videos, mostly each step of forelimb was followed by stimulated movement in lower limb, but sometimes

followed by none or two, even more. In addition, with telemetry recording, signals were transmitted wirelessly and stored by external computer. Our findings reported that motor neurons in the motor cortex were reconnected with the motor neurons in the spinal cord in awake guinea pigs.

The application of Motolink may be regarded as an electronic substitute of the corticospinal tract between the cortex and the spine. Our findings indicate that the Motolink may possibly be a novel therapeutic strategy for the recovery of functional locomotion of hind limbs in patients with spinal cord injury. This study demonstrated the results of connection by Motolink in pattern of single-to-single channel. In the future researches, it is possible to develop connection of Motolink in many-to-many channels for functional restoration of hindlimb. With multichannel technique, this circuit may benefit patients with spinal cord injury to partly restore motor functions.

1.3 Outline of the Thesis

Chapter one is introduction the background of this project, as well as the assumption, design and result of the research.

Chapter two reviews investigations on the following four research fields: existing and potential therapies of spinal cord injury, structure and locomotion control of motor system, application of neuroprostheses in the spinal cord injury, and introduction of intraspinal microstimulation. At the end of this chapter, based on literature reviews and critical thinking, a hypothesis is proposed for the study. Also the objectives of this study are given.

Chapter three describes experiments conducted in acute preparation rats, in which stimulation of the lumber spinal cord was triggered by signals from MGB, VC and AC.

Chapter four expresses chronic experiments performed on spinal guinea pigs, during which stimulation of the ventral spinal cord was triggered by neuronal signals from M1. Technique and result of telemetry Motolink are also showed in this chapter.

Chapter five is the conclusion and discussion together with suggestion for future experiments.

CHAPTER 2 LITERATURE REVIEW

2.1 Therapies of Spinal Cord Injury

Investigations on therapeutic strategies for the repair of spinal cord injury are explored extensively in the world wide. However many challenges of neuron regeneration in the spinal cord and functional recovery still lie in the therapeutic strategies. In recent decades, animal studies in the laboratory raise hope for treatment of SCI. These researches have been focused on four domains: neural stem cell transplantation, neurotrophic factors, gene therapy and neuroprosthesis. These four potential therapies are specified in the coming context: stem cells bridge the wound and boost the growth of neurons; neurotrophic factors block action of growth inhibitors and spur neurons growth across the injury site; gene therapy applies Nogo to produce growth inhibitor; and neuroprosthesis is a new pathway for the voluntary control of nonmuscular system.

In clinic, neuroprotection treatment with methylprednisolone is the only drug approved for use in patients with spinal cord injuries presently (Schwab 2002). Due to the effect of inhibiting inflammatory damage to spinal cord, Methylprednisolone has been completed large human trails and is the standard of care (Wickelgren 2002).

2.1.1 Stem cell transplantation

Researches on stem cell transplantation have been exploited as a progressive approach for the treatment of spinal cord injury. Stem cell transplantation is based on the regeneration ability of stem cells in the injured spinal cord in mammalian as they

do in lower vertebrates. Privat et al. reported that embryos raphe cells, which were injected into completely transected spinal cord, projected new axon into the gray matter regions of the host cord, and restored the sexual reflexes in rats (Privat, Mansour et al. 1989). Mouse embryonic stem cells that transplanted into rat's spinal cord after traumatic injury survived and differentiated, associated with hindlimb weight support and partial hindlimb coordination (McDonald, Liu et al. 1999). Electrophysiological recording showed that extracellular activity of graft neurons was evoked by stimulation on host neurons, which revealed the connectivity of host and grafts (Reier, Stokes et al. 1992). Rats and cats transplanted with fetal spinal cord (FSC) cells in acute and chronic injured spinal cord showed improvements in functions (Privat, Mansour et al. 1989; Anderson, Reier et al. 1991; Stokes and Reier 1992). In addition, single usage of olfactory ensheathing cells from olfactory lamina propria in the nose, or combined with other factors, contributed to the axonal regeneration and functional recovery of spinal cord injury in adult animals (Lu and Ashwell 2002; Fouad, Schnell et al. 2005).

Human neural stem/progenitor cells (NSPCs) transplanted into nonhuman primates survived, differentiated and enhanced the results of bar grip power and spontaneous motor activity of upper extremity (Iwanami, Kaneko et al. 2005). However, there is no Food and Drug Administration (FDA)-approved adult stem cell treatment or cure for spinal cord injury in clinical trials before 2006 (Smith, Neaves et al. 2006), until 2009 received clearance allowed the beginning of human safety testing of its stem cell treatment.

2.1.2 Neurotrophic factors and gene therapy

Axon regeneration of adult central nervous system has been a potential therapeutic strategy for SCI. Recently He Zhigang and his research group found that modulate intrinsic PTEN/mTOR pathway enhanced the regenerative ability of mouse adult retinal ganglion cells and corticospinal neurons, with functional recovery (Park, Liu et al. 2008; Liu, Lu et al. 2010; Park, Liu et al. 2010). These researches on regenerative ability of mouse adult corticospinal neurons lead to a great hope to functional recovery.

2.1.3 Neuroprosthesis

Artificially assistive devices have been developed for restoring function to paralyzed limbs and organs, which is neuroprosthetics. These devices can substitute the damaged function of motor, sensory and cognitive modality, such as the worldwide use of cochlear implants (Kessler 1999) and pacemakers (Brunner, Olschewski et al. 2004).

Previous studies on peripheral nerve system controlled functional electrical stimulation reported that patients with spinal cord injury gained functional improvement of hand by a hand neuroprosthesis called Freehand System (Fromm, Rupp et al. 2001; Smith, Mulcahey et al. 2001). This Freehand System used electromyographic activity from unimpaired muscles to control paralyzed muscles with functional electrical stimulation for functional recovery, bypassing around injury in the normal neural pathways.

2.1.4 Summary

The traumatic accidences are the chief cause that results in the spinal cord injury. Spinal cord injury leaves locomotion related areas of the brain intact but the supraspinal signals isolated from the spinal cord below the lesion, which disrupts the communication between brain and spinal cord.

There are four primary therapy of spinal cord injury: stem cells bridge the wound and boost the growth of neurons; neurotrophic factors block action of growth inhibitors and spur neurons growth across the injury site; gene therapy applies Nogo to produce growth inhibitor; and neuroprosthesis is a new pathway for the voluntary control of nonmuscular system.

2.2 Motor System

2.2.1 Organization of the motor system

The motor systems are organized in hierarchy from the cortex, brain stem to spinal cord. The spinal cord is at the lowest level of motor system, which contains neuronal circuits that control various reflexes and rhythmic movement such as locomotion and scratching. The brain stem is at the middle level of motor organization. Circuits mediating reflex of face and mouth and controlling movement of eyes and head are located here. Neurons in the brain stem receive signals from the forebrain and subcortical nuclei and project to the spinal cord. The cerebral cortex is the highest level in this hierarchical structure. The motor cortex contains the primary motor cortex, premotor area and secondary motor area. Axons from the primary motor cortex (M1) and some premotor areas project directly to the spinal cord through the corticospinal tract. All motor commands are transmitted to the motor neurons in the spinal cord or brain stem to innervate skeletal muscles. Muscle unit is the terminal of executing movement. The whole organization of motor system is demonstrated in Figure 2.1.

In the motor system, three levels function respectively but interactively: motor cortex and brain stem control, spinal cord relays and generates intrinsic locomotion, muscles execute action. Among many opinions on the relationship of the motor cortex, spinal cord and muslces, a popularly accepted opinion is “feedback remapping connectivity”, which is neither a simple relationship of many to many mapping nor one-to-one mapping (Graziano 2006). During locomotion, signals from the primary motor and premotor cortex are transmitted to the spinal cord by axons of upper motor neuron to activate motoneuron in the spinal cord. Lower motoneurons elicit groups of

muscle fibers for movement. Sensory information from visual system, efferent neurons in the dorsal root and axons in the spinal cord are transmitted to the sensory and motor areas of the brain. Such feedback includes evaluation of surrounding environment, position of the limbs and ground force in regard to obstacles and terrain.

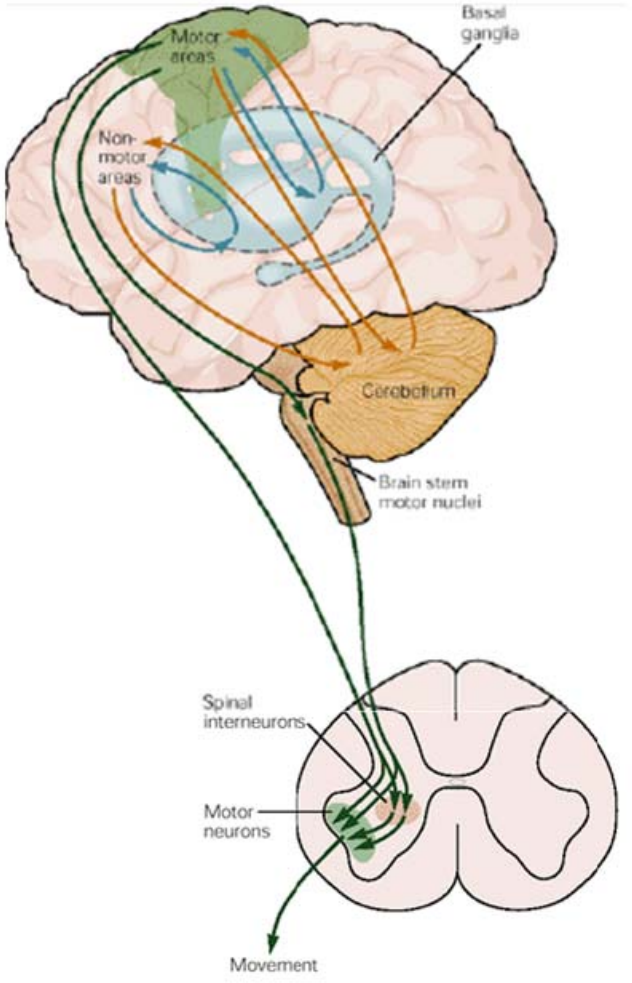

Figure 2.1 The motor systems have three levels of control: the spinal cord, brain stem, and forebrain. The motor areas of the cerebral cortex can influence the spinal cord either directly or through the descending systems of the brain stem. All three levels of the motor systems receive sensory inputs and are also under the influence of two independent subcortical systems, the basal ganglia and the cerebellum, through relay nuclei in the thalamus. (Source: Principles of Neural Science, Ver. 4th, Chapter 33, The organization of movement: 667, Figure 33-12).

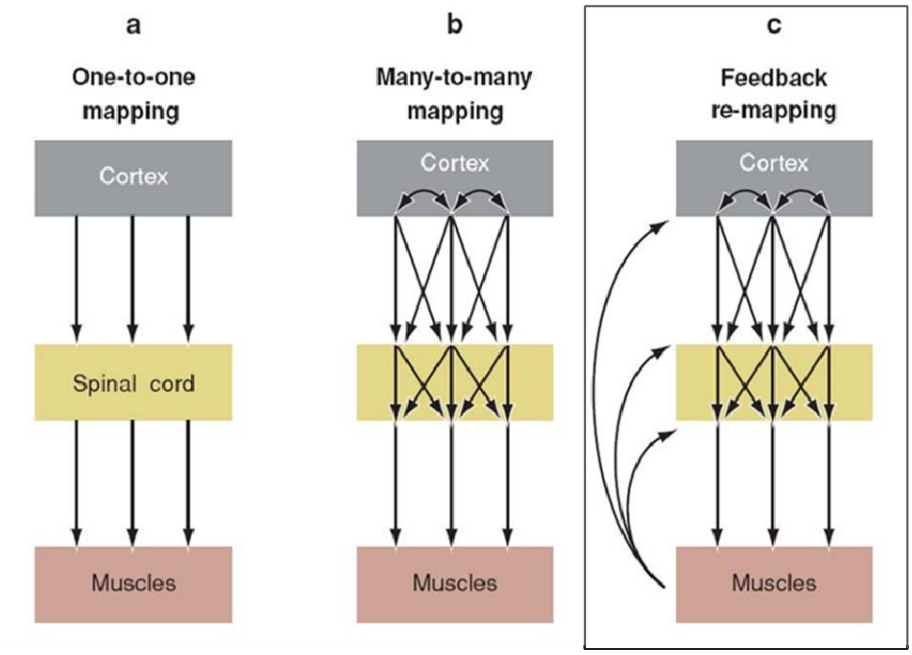

Figure 2.2 Three schematic views for the connectivity of cortex, spinal cord, and muscles. *A*: One-to-one mapping which is traditional but out of date. *B*: Many-to-many mapping in which internet insides cortex and spinal cord is proposed. *C*: Feedback remapping in which feedback from muscles and joints is incorporated. This model is the commonly acceptable explanation of the locomotion control of muscles by cortex and spinal cord, which highlighted by a frame (Graziano 2006).

2.2.2 Locomotion control of the primary motor cortex

2.2.2.1 Motor representation of the primary motor cortex

Motor cortex is divided in two subdivisions by their functions, which are area 4 and area 6 in Boddman (Figure 2.3). Area 4 refers to the primary motor cortex and area 6 is composed of premotor area (PMA) and supplementary motor area (SMA). Generally, the primary motor cortex is important for movement executing and the premotor and supplementary motor area are vital for planning and coordinating complex sequence of movement. A recent evidence in monkeys showed that the actual movement was represented in the primary motor cortex and the perceived movement was found in the ventral premotor cortex (Schwartz, Moran et al. 2004).

The primary motor cortex is located near the precentral gyrus of cortex in humans. The classical illustration of somatotopic representations in the primary motor cortex of human beings has been conveyed by the motor homunculus (Penfield and Rasmussen, 1950) and simiusculus (Woolsey et al., 1952) which resembles a little man. In homunculus and simiusculus, areas of representations are not proportion to the size of the body parts, but proportional to the precision of body control, such as the area representations of lips, face and fingers are the largest, whereas areas of the trunk and leg are relatively small. The hindlimb area locates medially, while the hand and face areas distribute laterally in the cerebral hemisphere, with partly overlapping of different representations (Figure 2.4).

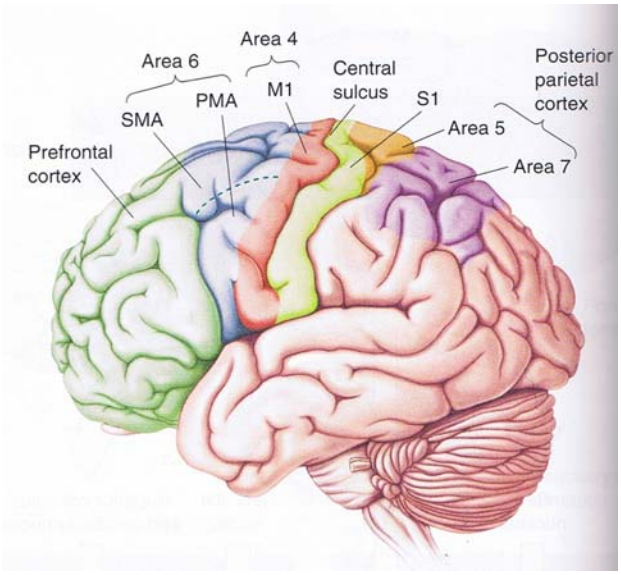

Figure 2.3 Division of areas in human cerebral cortex in Boddman. Area 4 is M1 and area 6 contains two parts: SMA and PMA. M1: primary motor cortex, PMA: premotor area, SMA: supplementary motor area.

In animals, the somatotopic representation of primary motor cortex such as forelimb and hindlimb areas is distributed in shape of irregular patches and the major subdivision is quite similar between animals (Neafsey, Bold et al. 1986). The primary motor cortex, premotor cortex and supplementary motor cortex have been investigated broadly in nonhuman primates (Strick and Preston 1978; Humphrey, Reed et al. 1983; Humphrey 1986; Nakayama, Yamagata et al. 2009). In addition, the organization of rats' primary motor cortex has been explored by several researchers (Donoghue and Wise 1982; Neafsey, Bold et al. 1986; Kleim, Barbay et al. 1998; Karl, Sacrey et al. 2008). Interestingly, the motor output organization in the rats is remarkably consistent with that of the primate (Neafsey, Bold et al. 1986).

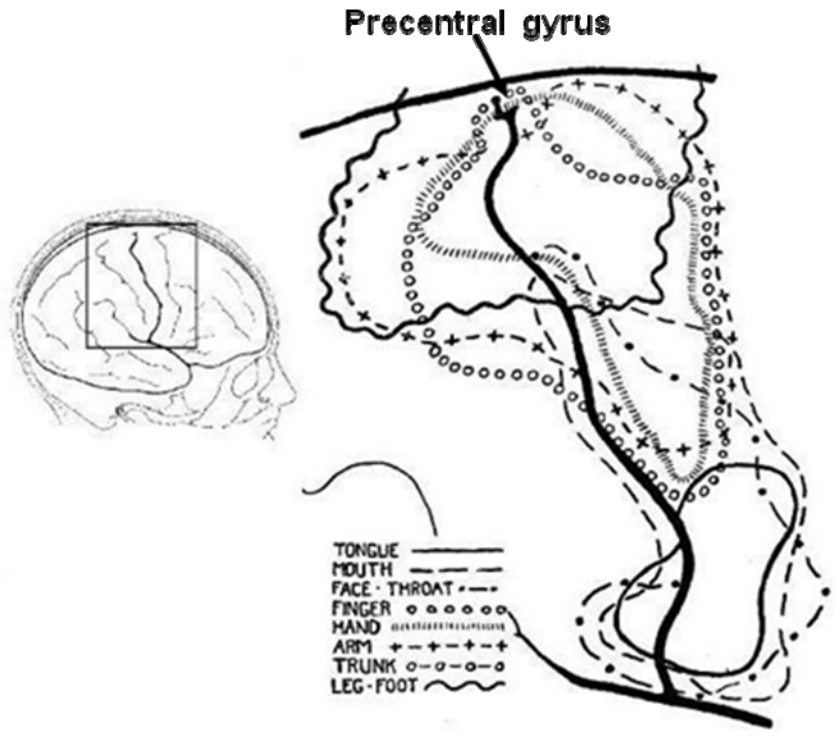

Figure 2.4 Outlines of the representation in the primary motor cortex of humans. Outlines encompass the total territory from which different body parts (fingers, mouth, hand, arm, trunk and leg-foot) were evoked. The thick black line in the middle of outlines represents the precentral gyrus. Note the convergence and overlap of distal and proximal representations. (Modified from (Schieber 2001).

Map of motor cortex averaged from 23 rats using epidural microstimulation (EMS) demonstrated that forelimb area is located in the anterior and lateral part of the frontal cortex, whereas hindlimb area is positioned more posterior and close to the midline (Molina-Luna, Buitrago et al. 2007). A general schematic of somatotopic representation in Figure 2.5 was derived from published papers, showing the relationship of M1, primary somatosensory cortex (S1), and secondary somatosensory cortex (S2) of rats, and the specific representations of different parts by intracortical electrical stimulation (ICMS). However, the representation of motor cortex contains not a simple map of the body's muscles and different action as once thought, but a

more complex and coordinating one for organization of the animal's behavioral repertoire (Graziano 2006). In Donoghue's study, they found the overlap zone of the distal hindlimb (HL) and a portion of the forelimb representations in M1 are located within a part of granular SI cortex (Donoghue, Leibovic et al. 1992).

The brain of guinea pig belongs to smooth brain tissue, which evolves at a higher level than that of other rodents. The cortical motor representations of the facial muscles in guinea pigs have been studied in recent year (Campos and Welker 1976; Lambert, Goldberg et al. 1985). In theory, the primary motor cortex of guinea pigs is similar to that of rats. However, the motor representations of vibrissa, forelimb, trunk and hindlimb in guinea pigs have not been investigated. In our study, neural activities of the motor cortex, especially the hindlimb region of the motor cortex, must be collected to produce activation of the lower motor neurons. Therefore, the hindlimb representations of the primary motor cortex must be identified initially in the pilot study.

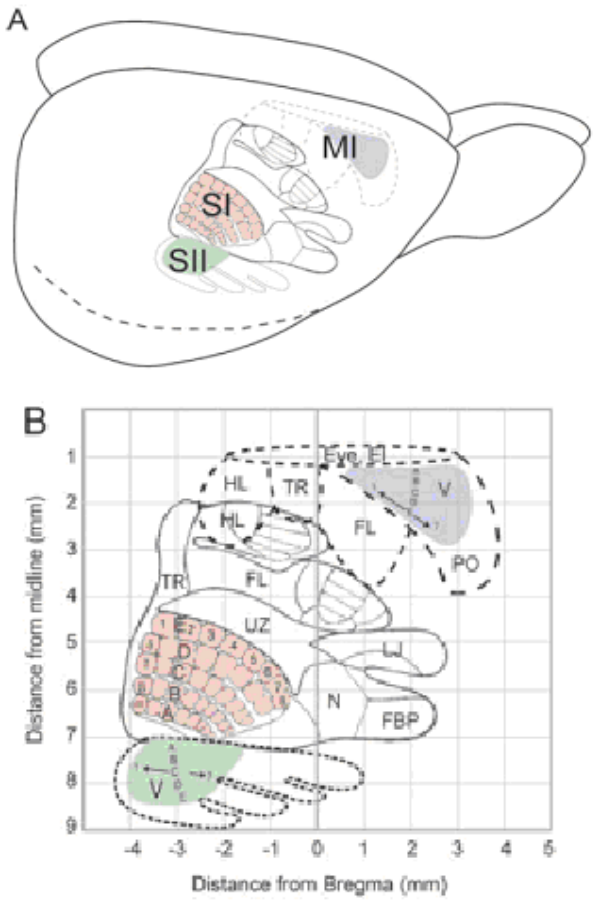

Figure 2.5 Motor representations in primary motor cortex (MI), primary somatosensory cortex (SI), and secondary somatosensory cortex (SII) of rats. A. The cartoon representation indicates the position of MI (blue), SI (pink), and SII (green) in the right cerebral hemisphere. **B.** The outlines of the MI, SI, and SII maps were adapted from several published maps of these areas. The horizontal axis shows the anterior-posterior distance to Bregma. The vertical axis shows distances from the midline. A–E, whisker rows; 1–8, whisker arcs; El, eyelid; FBP, furry buccal pad; FL, forelimb; HL, hindlimb; LJ, lower jaw; N, nose; PO, perioral; TR, trunk; UZ, unresponsive zone; V, vibrissae. (Leergaard, Alloway et al. 2004)

2.2.2.2 Locomotion control of the primary motor cortex

The fifth layer of the primary motor cortex contains giant pyramidal motor neurons that project long axons to the contralateral motor neurons in the anterior horn of the spinal cord, which forms the corticospinal tract (CST). These big pyramidal

cells are called as Betz cells, which Vladimir Alekseyevich Betz described and published in his work in 1874. They are mainly located in laminar Vb of the primary motor cortex (Meyer, 1987) and account for around 10 percent of the population of pyramidal neurons in layer Vb per hemicerebrum (Rivara, Sherwood et al. 2003). The Betz cells and their long axons are known as upper motor neuron (UMN). By the use of cortical microstimulation, low threshold (≤60 μA) zones were obtained nearby the layer V which containing large pyramidal cells (Donoghue, Leibovic et al. 1992).

In principle, single neurons in the motor cortex can contribute to different behavior through their broad tuning to spectacular and multijoint actions (Graziano 2006). Early in 1980s, Georgopoulos and his colleagues reported that motor cortical discharge was tuned to the direction of movement as monkeys made two-dimensional and three-dimensional reaching movements (Georgopoulos, Kalaska et al. 1982; Georgopoulos, Kalaska et al. 1982; Georgopoulos, Kettner et al. 1988; Kettner, Schwartz et al. 1988; Schwartz, Kettner et al. 1988). After that, spiking activity of primary motor cortex and movement kinematics has been well studied in able-bodied nonhuman primates. Neuronal population of the primary motor cortex had been observed to code the direction, speed and position of movement to visual targets in various tasks. Activity of cortical populations visualized by approach of population vectors calculated from large neuronal populations predicted the trajectory of monkey's hand as it drew spirals (Schwartz 1994). The speed of movement was found to be represented in the single cell activity of arm area in the motor cortex during monkey's reaching task, but the effect of speed on cortical and muscle activity was different (Moran and Schwartz 1999). Hand position and velocity were involved in the information of cortical activity of motor cortex during visually guided tracking

(Paninski, Fellows et al. 2004). Most interestingly, these movement parameters of neurons discharge have been discovered to occur in temporal sequence: direction related discharge appeared firstly, then target position occurred and followed by movement distance (Fu, Flament et al. 1995). Therefore, much of the cortical neural activities is informative and can be applied in controlling neuroprosthetic effectors in individuals with physical disability, which helps to restore or replace the function of paralyzed hands or legs with their volitional control.

The basic motor pattern for stepping in mammals is generated by the neuronal circuits within the spinal cord. In voluntary movement, besides the control by central pattern generator, descending pathways that contain the motor cortex, cerebellum and the brain stem are involved in fine control of stepping activity. Rhythmical activities from neurons in all these regions have been recorded actively during normal locomotor pattern. The motor cortex essentially functions during visually guided walking. The change of activity in cortical neurons is modulated by input from visual cortex. When a normal cat walked over obstacles fixed to a moving treadmill belt, neurons in the motor cortex increased their peak discharge rate to adapt stepping movements, which was associated with enhanced muscle activity in forelimb flexors (Drew 1988). Neurons related to hindlimb musculature also showed significant increase in firing rate when cats performed the same task as stepping over obstacles attached on the belt of treadmill (Widajewicz, Kably et al. 1994). Cortical control has been proved to be indispensable for the structure and the timing of hindlimb locomotion in cats (Bretzner and Drew 2005).

2.2.2.3 Plasticity of the primary motor cortex after spinal cord injury

Many treatments for restoring the lost function of patients with spinal cord injury assume that the brain motor cortex is relatively intact (Carmena, Lebedev et al. 2003; Dobkin and Havton 2004; Friehs, Zerris et al. 2004). The intact motor cortex makes it possible to access voluntary cortical signals for the use of cortical neural prosthesis (Wessberg, Stambaugh et al. 2000; Nicolelis 2001; Nicolelis 2003). These therapies based on the hypothesis that motor cortex maintained efficiently excitable to respond to motor attempts and generate signals for driving extremity movement after SCI. According to Shoham's study, reorganization of the motor system in subjects with SCI did not affect attempt-related activation and the somatotopic activation closely followed that of normal (Shoham, Halgren et al. 2001). Relationship between spiking activity in primary motor cortex and intended movements kinematics was found to be correlated in two humans with tetraplegia (Truccolo, Friehs et al. 2008). Their results indicated that M1 significantly tuned to velocity and position of intended hand movement after years of loss of descending motor pathways.

However, other teams of scientists have come up with conflicting evidences. They believed the representation motor cortex can shift to adopt able-parts of the body following paraplegia or tetraplegia to adapt to disabilities, leading to remapping of the motor cortex. The spiking model also changes due to loss of sensory input from afferent nerves below the lesion. In the model of feedback remapping in Figure 2.2, the organization of motor cortex can adapt to exercise training or loss of nerves afferent gradually (Sanes and Donoghue 2000) , but the adaptation do not occur instantly after brain or peripheral motor nerve lesion (Kakei, Hoffman et al. 1999; Graziano, Patel et

al. 2004). Green and Lacouse showed that the amplitude and latency of movement-related potentials in primary motor cortex were reduced and the variance became larger than the control group in individuals with chronic spinal cord injury (Green, Sora et al. 1999; Lacourse, Cohen et al. 1999). Moreover, Cramer et al. found that, though many features of brain motor function were preserved, volume of activation was much reduced, abnormal activation patterns and abnormal processing were present in S1 during imagined movement (Cramer, Lastra et al. 2005). The abnormalities of M1 and S1 implied that the plasticity of motor cortex after SCI should be noticed.

2.2.3 Locomotion control of the spinal cord

2.2.3.1 The anatomical organization of the spinal cord

The spinal cord is part of central nerve system which communicates between the brain and other part of the body. It contains neural circuits that can coordinate reflexes and central pattern generator that can produce rhythmic locomotor activity. By the great work done on leg reflexes controlled by spinal cord motor neurons in cats, Sherrington got the Nobel Prize (Kinsbourne 1980). The spinal cord is divided into white matter and gray matter. In white matter there are axons which contain descending and ascending axons of sensory neurons in dorsal root, that are grouped in numerous tracts. Gray matter is comprised of the motor neurons and their dendrites, interneurons, propriospinal neurons and glia cells. There are total 32 spinal segments in human spinal cord, including 7 cervical segments, 12 thoracic segments, 5 lumbar segments, 5 sacral segments and 3 coccygeal segments. Nevertheless, segment organization of the spinal cord of rats is different from that of humans. There are total

34 spinal segments in rats' spinal cord, including 8 cervical segments, 13thoracic segments, 6 lumbar segments, 4 sacral segments and 3 coccygeal segments. Representation of different hindlimb muscles in the spinal cord of cats was investigated through electrical microstimulation and found mainly in the lumbar and sacral segments (Mushahwar and Horch 2000). However, the representation of the lumbar spinal cord in guinea pigs is still not clear.

2.2.3.2 Locomotion control of the spinal cord

The spinal motor neurons execute movement. Their axons exit out of the spinal column to innervate muscles. The cell bodies of motor neurons are clustered in the ventral horn of the spinal cord, which from longitudinal column extending from one to four segments (Figure 2.6). The spatial distribution of motor pools is organized proximaldistally. Motor neurons innervating more proximal muscles located medially while motor neurons eliciting most distal muscles lie most laterally.

Central pattern generator (CPG) is a genetic locomotor network inside the spinal cord which contributes to the coordination and synergy of different levels in the spinal cord. There are many evidences of central pattern generator for generating rhythmic locomotion in the spinal cord of non-human vertebrates (Grillner 1985) and human (Calancie, Needham-Shropshire et al. 1994; Dimitrijevic, Gerasimenko et al. 1998; Maegele, Muller et al. 2002). Kittens with complete transection of the spinal cord at T10-12 could perform alternating limb movements and late walking on a treadmill few days after surgery (Forssberg, Grillner et al. 1980; Forssberg, Grillner et al. 1980), which happened more easily in young cats rather than adult cats. Spinal cats trained to

walk on the treadmill daily can step more successfully than untrained cats. Following the lesion at thoracic or higher levels, the lumbosacral spinal cord is isolated from upper central nerve system and is found to reorganize with new physiological function which is nearly the same as normal animal does. The loss of supraspinal input inevitably makes some changes in the remaining synapses of interneurons and propriospinal neurons, thus results in the spinal cord caudal to the lesion forming a new spinal cord to executed independent stepping or standing. Furthermore, spinal cats learned full weight support standing on one hindlimb or both hindlimbs that received daily stand training of the hindlimbs over a period of 12 weeks (De Leon, Hodgson et al. 1998). The spinal cord of adult complete spinal rats and cats could learn to step and stand if received training for these specific tasks. Humans with spinal cord injury also responded to locomotor training in subjects with an incomplete injury. All of these observations clearly demonstrate the existence of the central pattern generator in the lumbosacral spinal cord after complete transaction.

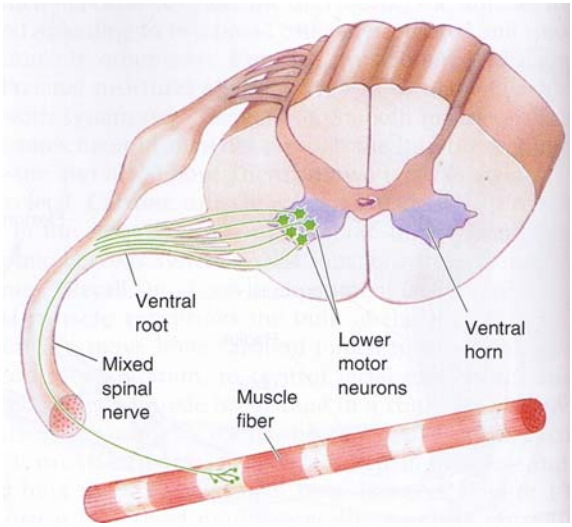

Figure 2.6 Muscle fibers are innervated by lower motor neurons. (Source: Principles of Neural Science, Ver. 4th, Chapter 33, The organization of movement: 667, Figure 33-12)

2.2.4 Summary

The motor systems are organized in hierarchy from the cortex, brain stem to spinal cord. Feedback remapping in which feedback from muscles and joints is incorporated is commonly acceptable model of the locomotion control of muscles by cortex and spinal cord. The primary motor cortex is important for movement executing and the premotor and supplementary motor area are vital for planning and coordinating complex sequence of movement. The fifth layer of the primary motor cortex contains giant pyramidal motor neurons that send long axons to the contralateral anterior horn of the spinal cord, which forms the corticospinal tracts. The primary motor cortex of guinea pigs is similar to that of rats, but it has not been investigated experimentally.

Single neurons and neuronal populations in the motor cortex contribute to different behavior through tuning to the direction, position and speed of movements. Much of the cortical neural activities is informative and can be applied in controlling

neuroprostheses. Remapping of the motor cortex occurs following paraplegia or tetraplegia to adapt to disabilities.

The basic motor pattern for stepping in mammals is generated by the neuronal circuits within the spinal cord. However, the motor cortex essentially functions during visually guided walking. Central pattern generator is a genetic locomotor network inside the spinal cord which contributes to the coordination and synergy of different levels in the spinal cord. The representation of the lumbar spinal cord in guinea pigs is still not clear.

2.3 Neuroprostheses

2.3.1 Brain computer interface

Brain computer interfaces (BCIs) are fundamentally neuroprosthetic which connect the brain with computer system. Brain computer interfaces allow control of computers or external devices with regulation of brain activity in a different way (Birbaumer 2006). Depending not on nerves and muscles, BCIs provide the brain with a new communication and control pathway to the non-muscular system for conveying command signals to the outside world (Wolpaw, Birbaumer et al. 2002). The primary goal of BCI researches is to enable users with motor disabilities such as spinal cord injury, brainstem stroke, muscular dystrophy, or amyotrophic lateral sclerosis (ALS) and other muscular diseases, to communicate with others, control a robotic arm, or a neuroprosthesis to restore their lost motion function (Wolpaw and McFarland 2004).

Chronic microelectrode arrays, mathematics algorithms of extraction and neuroprosthesis to engage effectors are three vital conditions for brain computer interface (Schwartz 2004). The overview of the design and effectors of a BCI system containing these three essential elements is demonstrated in Figure 2.7 A. According to the different recording methods of acquiring electrophysiological signals, BCI systems can be classified to three categories: electroencephalographic (EEG)-based BCIs, electrocorticographic (ECoG)-based BCIs, and intracortical BCIs (Wolpaw, Loeb et al. 2006; Daly and Wolpaw 2008). The detail locations of recording sites for EEG activity, ECoG activity, and neuronal action potentials (spikes) or local field potential (LFP) are shown in Figure 2.7 B. Based on whether there is damage to brain tissue, BCIs is divided into two types: noninvasive and invasive BCIs. EEG activity is

recorded at the scalp, which is noninvasive and easily to be acquired but has relatively limited topographical resolution and frequency range; ECoG activity is recorded at the cortical surface which has better resolution and frequency range than EEG activity; neuronal action potentials or LFP have the highest resolution but entail invasively implantation of multichannel electrodes array which has significant risk (Wolpaw, Loeb et al. 2006). ECoG and intracortical BCI are both invasive.

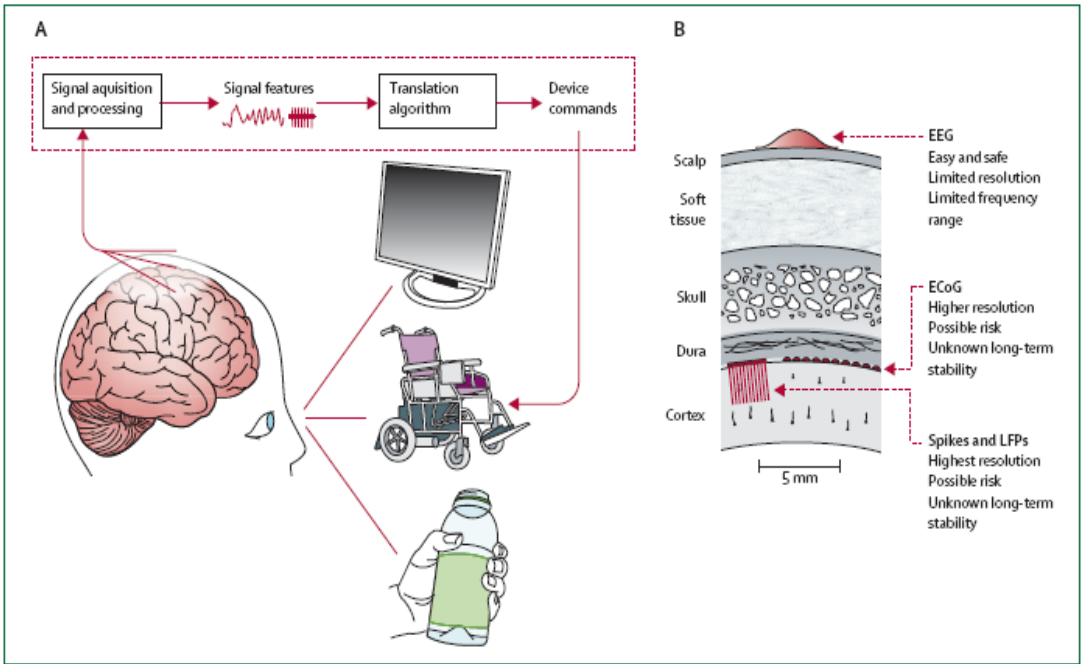

Figure 2.7 Overview of components of a BCI system. (A) Design and operation of a BCI system. Electrophysiological signals are obtained from the scalp, the cortical surface, or within the brain, and signal features are analysed, such as amplitudes of event-related potentials, EEG rhythms, or firing rates of single neurons. These features are translated into commands that operate an output device, such as a word-processing program, a wheelchair, or a neuroprosthetic limb. (B) Recording locations for brain activity used by BCI systems. (Daly and Wolpaw 2008)

In recent years, the rapid development of Brain Computer Interfaces is contributed to the following four factors: the increased understanding of signal features

of the brain, the recognition of activity-dependent plasticity, the universal availability of hardware and software for signal processing, and the increased social interest and potential needs (Daly and Wolpaw 2008). Application of the brain computer interface developed from single dimension to multi dimensions control, also from simple to complex action. An example of one-dimensional control was reported by John K. Chapin and his colleagues. In their study, rats were trained to used brain-derived signals to position a robot arm and obtain water by pressing a lever (Chapin, Moxon et al. 1999). Lately, scientists showed noninvasive BCI provided humans, with or without spinal cord injury, the two-dimensional control of cursor movement on computer screen (Wolpaw and McFarland 2004). Most recently, Meel Velliste et al. presented monkeys used their cortical activity from the primary motor cortex to control a robotic arm for feeding itself in a three-dimensions movement with physical interaction, also to control a gripper on the end of the robotic arm proportionally (Velliste, Perel et al. 2008). More surprising, a tetraplegic human subject after three years training by neuromotor prostheses (NMPs) could successfully use neuronal ensemble activity to control the cursor of a computer, opened a simulated e-mail, operated television, open and close a prosthetic hand, and performed elementary actions with a multi-jointed robotic arm (Hochberg, Serruya et al. 2006). NMPs are based on neural prosthetics from the motor cortex and include recording electrodes as well as stimulating electrodes to achieve prosthetics. Brain Gate (Cyberkinetics, Inc.) is a commercial NMPs system which has been under developing for people with tetraplegia to perform basic tasks.

Invasive brain-computer interface requires implantation of electrodes array into the outer layer of the cortex to access neuronal signals. With respect to invasive BCIs,

there are many investigations in animals or humans. Kennedy P.R. al. illustrated, with especially cortical implanted electrodes, a patient learned to control a computer cursor to produce synthetic speech and typing by brain-computer interface devices system (Kennedy, Bakay et al. 2000). Wessberg J. et al. suggested that simple real-time ensembles of cortical neurons predict one- and three-dimensional arm movement trajectories, and achieve complex prosthetic robot arm movements in primates (Wessberg, Stambaugh et al. 2000). More complete reaching task including elbow and wrist movements may be achieved through a high-resolution BCI system by using at least six independent control signals (Leuthardt, Miller et al. 2006). A neuroprosthesis which was implemented by autonomous electronic circuitry direct transformed cortical activity to muscle stimulation, led to the restoration of goal-directed movements to a transiently paralysed arm on Macaca nemestrina monkeys, and generated bidirectional wrist torques (Moritz, Perlmutter et al. 2008).

2.3.2 Noninvasive brain computer interface

Related to noninvasive BCI, skull and scalp recorded electroencephalographic activity is one type of noninvasive BCI technology. Movement-related information from these EEG signals, including frequency, amplitude and evoked potentials, are extracted to allow human subjects to achieve basic task such as control a light switch (Bormane, Patil et al. 2009), and even more complex tasks including moving a one-dimensional computer cursor (Wolpaw, McFarland et al. 1991), controlling two-dimensional movement of computer cursor by two channels of EEG activity (Wolpaw, McFarland et al. 1991; Wolpaw and McFarland 1994), spelling out messages and

sentence-construction tasks (Neuper, Muller-Putz et al. 2006), or controlling an upper extremity grasping neuroprosthesis (Mahmoudi and Erfanian 2002). Although it is commonly recognized that recording individual action potentials requires an invasively place the recording electrodes, Wolpaw and McFarland had demonstrated third times the ability of extracting two independent cortical control signals from noninvasive skull-recorded EEG. Wolpaw and McFarland demonstrated that two significantly different control signals could be extracted from EEG activity recorded from human subjects. In 2003, they showed that a well-trained subject used the mu (8-12 Hz) or beta (18-25 Hz) rhythm amplitude of electroencephalogram activity to perform a one-dimensional, center-out cursor task (McFarland and Wolpaw 2003). In 2004, they were able to achieve two-dimensional control of a computer cursor through scalp-recorded EEG and an adaptive algorithm, with results comparable to those obtained from monkeys implanted with multiple cortical recording electrodes (Wolpaw and McFarland 2004).

All though some scalp-based EEG-based BCIs have been applied successfully in people with forms of paralysis, these systems have some disadvantages, such as requiring the subject to do significant learning before performance, concentration to the exclusion of other actions, and daily scalp sensor application, though it has no requirement of surgical placement of the sensor and less damage to the intact brain. Therefore, the application of grasp neuroprostheses have been applied to limited tetraplegic patients at present due to the lack of adequate control interfaces. In contrast, the direct invasive BCI requires less pre-learning beyond filter creation, and can be used during natural activities such as speech. It is also plausible that invasive BCI could be recorded from large scale of the cortex, which contains more accurate

information so that parallel commands could be derived simultaneously from multiple sensors each in separate cortical regions. If achieved, relatively independent action potentials could efferent from arm and leg areas bilaterally to allow for the reanimation of paralyzed limbs via functional electrical stimulation devices (Hochberg, Serruya et al. 2006).

2.3.3 Summary

Brain computer interfaces allow control of computers or external devices with regulation of brain activity, which is not dependent on the peripheral muscle system. Chronic microelectrode arrays, mathematics algorithms of extraction and neuroprosthesis to engage effectors are three vital conditions for brain computer interface. Based on whether there is damage to brain tissue, BCIs is divided into two types: noninvasive and invasive BCIs. EEG activity is recorded at the scalp, which is noninvasive and easily to be acquired but has relatively limited topographical resolution and frequency range; ECoG activity is recorded at the cortical surface which has better resolution and frequency range than EEG activity; neuronal action potentials or LFP have the highest resolution but entail invasively implantation of multichannel electrodes array which has significant risk. A tetraplegia human subject after three years training by neuromotor prosthesis could successfully use neuronal ensemble activity to control the cursor of a computer, opened a simulated e-mail, operated television, open and close a prosthetic hand, and performed elementary actions with a multi-jointed robotic arm.

All though some scalp-based EEG-based BCIs have been applied successfully in people with forms of paralysis, these systems have some disadvantages. Though risk existing, invasive BCI could be recorded from large scale of the cortex and contains more accurate information to perform more complicated activity in both upper and lower, right and left limbs, as well as requiring less pre-learning.

2.4 Intraspinal Microstimulation

2.4.1 Functional electrical stimulation

Functional electrical stimulation (FES) commonly applies electrical stimulation on various levels of nervous system and muscles in order to restore physical functions such as locomotion, hearing and micturition et al. It is a replacement of the lost descending motor commands with artificial stimuli. Many successful FES systems have been implanted in a number of populations, for example, the heart pacemaker for heart disease patients (Brunner, Olschewski et al. 2004), the cochlear stimulator for deaf individuals (Kessler 1999), the deep-brain stimulators for headache and Parkinson's disease individuals (Benabid, Wallace et al. 2005), the bladder stimulator for micturition (Brindley, Polkey et al. 1982; Rijkhoff 2004), the phrenic nerve stimulator for respiration (Elefteriades and Quin 1998), and the vagal nerve stimulator for care of epilepsy (Shaffer, Jackson et al. 2005). Neural prostheses (NPs) are another form of FES that can restore lost functions. Since 1963 over 40000 people have been implanted with implantable NPs to restore hearing, bladder control and respiration. Surface NPs for muscle contraction are very common in clinics of rehabilitation and many homes currently (Prochazka, Mushahwar et al. 2001). In addition, deep brain stimulation (DBS) has been carried out to control intractable pain and in individuals with chronic pain syndromes (Feldman 1979; Kumar, Toth et al. 1997; Wallace, Ashkan et al. 2004). Epidural spinal cord stimulation (ESS) has been applied to control spasticity, tremor and rigidity in chronic spinal cord injured patients (Nomura, Fukuuchi et al. 1995; Midha and Schmitt 1998).

There are several kinds of FES for restoring lower-limb movements. However, the basic disadvantage of muscle functional electrical stimulation is that large numbers of muscles must be activated and many electrodes must be implanted into them for restoration of functional movement as walking and standing (Marsolais and Kobetic 1987). The change of status of muscles and easy to become fatigue are all challenging problems of FES in muscles.

2.4.2 Intraspinal microstimulation

Intraspinal microstimulation (ISMS) is a novel approach of FES that stimulates the spinal cord via microelectrodes directly. It is an alternative therapy of restoring mobility, which takes advantage of the spinal cord locomotor circuits, and of bladder control (Rijkhoff 2004). Two characters of the spinal cord contribute to the application of intraspinal microstimulation. Firstly, the spinal cord is distant from contracting muscles, thus leading the electrodes implanted not to be subjected to damaging stresses and strains during muscles movement. Secondly, the lumbo-sacral spinal cord which contains motor neurons innervating lower extremities muscles is compressed and allows implanting electrodes in a relatively small and protected region (Mushahwar, Collins et al. 2000). Mushahwar identified the representative location of quadriceps, tibialis anterior and triceps surae/plantaris muscles in the spinal cord of feline by electrically stimulating motor neurons in the ventral lumbo-sacral spinal cord. Graded control of individual muscles and muscle groups were also obtained, which indicates the application of functional neuromuscular stimulation (Mushahwar and Horch 2000). Detailed electrophysiological mapping of the lumbosacral region of the spinal cord

controlling leg movements in deeply anaesthetized adult cats demonstrated that intraspinal microstimulation can produce smooth and graded single joint movements, with a nearly normal order of recruitment of motor units (Mushahwar and Horch 2000).

Bipedal locomotor-like stepping and feedback-controlled movements of the hindlimb could be achieved by microstimulation of the spinal cord in anesthetized or chronically implanted cats (Mushahwar, Collins et al. 2000; Mushahwar, Gillard et al. 2002; Mushahwar, Aoyagi et al. 2004). The electrode implantation procedure used in chronically implanted experiments is illustrated in Figure 2.8. The microwires were individually inserted through the dorsal surface of the spinal cord and targeted at the ventral horn. The technology of fixation has successfully maintained the implanted microwires stably in the spinal cord and the stimulation through these electrodes produced stable response over time and controllable movement of hindlimbs (Mushahwar, Collins et al. 2000). Bilaterally coordinated stepping of the hindlimbs could be obtained by at least two stimulating electrodes in each side of the spinal cord under pentobarbital anesthesia (Mushahwar, Gillard et al. 2002). As regarding to the strategies of generating prolonged standing, closed-loop control combined with interleaved intraspinal microstimulation could achieve prolonged electrical stimulation standing in anesthetized cats compared to open-loop control and non-interleaved intramuscular stimulation (IM-S) (Lau, Guevremont et al. 2007).

Sustained electric stimulation of the epidural posterior lumbar spinal cord also induced stepping-like movements in subjects with chronic, complete spinal cord injury (Minassian, Jilge et al. 2004; Gerasimenko, Ichiyama et al. 2007; Lavrov, Dy et al. 2008). To examine mechanisms underlying the stepping-like movements activated by electrical epidural stimulation of posterior lumbar cord structures, Minassian K and his

colleagues have demonstrated epidural stimulation of the posterior lumbosacral cord in a segmental-selective way (Minassian, Jilge et al. 2004).

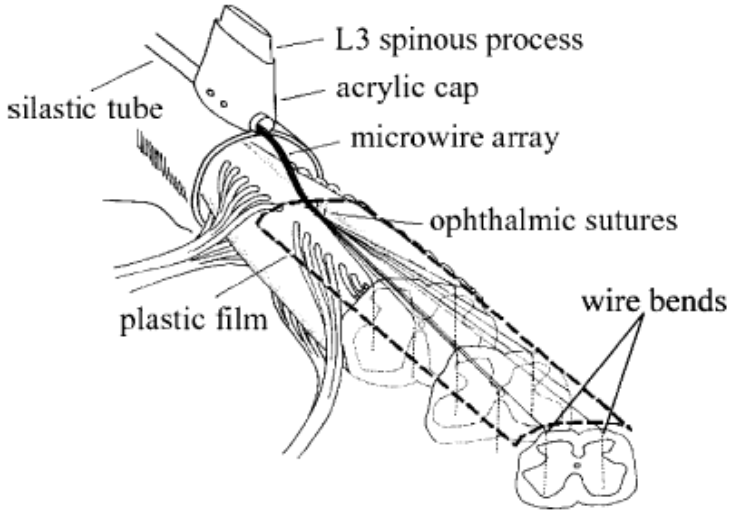

Figure 2.8 The schematics of implantation and fixation of stimulation microwires in the spinal cord. Five pairs of microwires were inserted in the ventral horn of the spinal cord. The array of microwires is tunneled in a silastic tube and attached to the L3 spinous process with an acrylic cap. Plastic film is placed over the array to separate the array layer from superficial tissues. The microwires were spaced 1.7–2.1 mm from the midline, 3–4.2 mm deep, and 2–3 mm apart along the longitudinal of the lumbar enlargement (Adapt from (Saigal, Renzi et al. 2004).

2.4.3 Summary

Functional electrical stimulation is a replacement of the lost descending motor commands which applies electrical stimulation on various levels of nervous system and muscles in order to restore physical functions such as locomotion, hearing and micturition et al. Neural prostheses for muscle exercise are very common in clinics of rehabilitation and many homes currently. Deep brain stimulation and epidural spinal cord stimulation has been applied to control intractable pain and spasticity.

Intramuscular stimulation for restoring lower-limb movements has disadvantage of the fact that large numbers of muscles must be activated and numerous electrodes must be implanted into the muscles for restoration of functional movement as walking and standing(Marsolais and Kobetic 1987). In addition, the easily changeable status of muscles and muscle fibers becoming fatigue in short period are problems of intramuscular stimulation.

Intraspinal microstimulation is a novel approach of FES that stimulates the spinal cord via microelectrodes directly. Two characters of the spinal cord contribute to the application of intraspinal microstimulation. Firstly, the spinal cord is distant from contracting muscles, thus leading the electrodes implanted not to be subjected to damaging stresses and strains during muscles movement. Secondly, the lumbo-sacral spinal cord which contains motor neurons innervating lower extremities muscles is compressed and allows implanting electrodes in a relatively small and protected region.

Bipedal locomotor-like stepping and feedback-controlled movements of the hindlimb could be achieved by microstimulation of the spinal cord in anesthetized or chronically implanted cats. The technology of fixation in chronic experiments has

successfully maintained the implanted microwires stably in the spinal cord and the stimulation through these electrodes produced stable response over six months. Closed-loop control combined with interleaved intraspinal microstimulation could achieve prolonged electrical stimulation standing in anesthetized cats.

2.5 Summary

There are four domains of therapy of spinal cord injury: neural stem cell transplantation, neurotrophic factors, gene therapy and neuroprosthesis. The motor systems are organized in hierarchy from the cortex, brain stem to spinal cord. Feedback remapping of the locomotion control of muscles by cortex and spinal cord is commonly acceptable opinion. The primary motor cortex is important for movement executing and the premotor and supplementary motor area are vital for planning and coordinating complex sequence of movement. The fifth layer of the primary motor cortex contains giant pyramidal motor neurons that send long axons to the contralateral anterior horn of the spinal cord, which forms the corticospinal tracts. Single neurons and neuronal populations in the motor cortex contribute to different behavior through tuning to the direction, position and speed of movements. Much of the cortical neural activities is informative and can be applied in controlling neuroprostheses. Remapping of the motor cortex occurs following paraplegia or tetraplegia to adapt to disabilities.

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Brain computer interfaces allow control of computers or external devices with regulation of brain activity, which is not dependent on the peripheral muscle system. Chronic microelectrode arrays, mathematics algorithms of extraction and

neuroprosthesis to engage effectors are three vital conditions for brain computer interface. Based on whether there is damage to brain tissue, BCIs is divided into two types: noninvasive and invasive BCIs. EEG activity is recorded at the scalp, which is noninvasive and easily to be acquired but has relatively limited topographical resolution and frequency range; ECoG activity is recorded at the cortical surface which has better resolution and frequency range than EEG activity; neuronal action potentials or LFP have the highest resolution but entail invasively implantation of multichannel electrodes array which has significant risk.

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successfully maintained the implanted microwires stably in the spinal cord and the stimulation through these electrodes produced stable response over six months. Closed-loop control combined with interleaved intraspinal microstimulation could achieve prolonged electrical stimulation standing in anesthetized cats.

2.5.1 Limitation of previous researches

In animals, the somatotopic representation of primary motor cortex such as forelimb and hindlimb areas is distributed in shape of irregular patches and the major subdivision is quite similar between animals. In theory, the primary motor cortex of guinea pigs is similar to that of rats, but it has not been investigated experimentally.

All though some scalp-based EEG-based BCIs have been applied successfully in people with forms of paralysis, these systems have some disadvantages, such as requiring the subject to do significant learning before performance, concentration to the exclusion of other actions, and daily scalp sensor application, though it has no requirement of surgical placement of the sensor and less damage to the intact brain. Therefore, the application of EEG-based BCIs has been limited. In contrast, though existing risk, invasive BCI could be recorded from large scale of the cortex and contains more accurate information to perform more complicated activity in both upper and lower, right and left limbs, as well as requiring less pre-learning.

In the intramuscular FES for restoring lower-limb movements, large numbers of muscles must be activated and numerous electrodes must be implanted into the muscles for restoration of functional movement as walking and standing(Marsolais and

Kobetic 1987). In addition, the easily changeable status of muscles and muscle fibers becoming fatigue in short period are problems of intramuscular stimulation.

2.5.2 Assumption of this study

It is natural that subject intends to do the desired movement of walking and standing even they have motor disabilities. However, functional electrical stimulation applied in the spinal cord for restoration of functional limb locomotion after spinal cord injury often uses artificial control signals to activate motor neurons in the spinal cord so as to innervate muscles (Saigal, Renzi et al. 2004; Bamford, Putman et al. 2005; Guevremont, Renzi et al. 2006; Lau, Guevremont et al. 2007), rather than uses volitional signals from the motor cortex. Mushahwar and his colleagues proposed theoretically that the movement of stepping after SCI could be controlled with one or two independent cortical signals by using an intraspinal microstimulation with assistive computer system (Mushahwar, Guevremont et al. 2006). The motor tasks to be intended are the imagination of modulation and adjustment of hindlimb movement. Since the neural activity of motor cortex can be used to control external devices, and functional electrical stimulation can be used to activate spinal cord motor neurons, we sought to test the feasibility that the neural activity of motor cortex is used to control functional electrical stimulation which thus activates the spinal motoneurons below the injury in spinal cord injured animals. Therefore, controlling FES by signals from hindlimb area of primary motor cortex is assumed to be test in this study.

Base on the fact that voluntary commands from the motor cortex cannot deliver down through the spinal cord to the site below the lesion in complete spinal cord injury, we try to build up a new pathway to substitute the damaged one. Therefore, the assumption of this study is to reconnect motor neurons in the motor cortex with motoneurons in the lumbar spinal cord, in order to innervate related muscles by

electrical pathway in animals with transected spinal cord. In other word, we want to apply personal thoughts to control paralyzed body directly. In this new pathway we change the output from the brain to the spinal cord. The flowchart in illustrates this artificial substitute pathway, which is named “Motolink”.

For this purpose, we develop a set of circuit to implement signal picking-up and intraspinal functional electrical stimulation, for generating hindlimb movement in animals with spinal cord injury. It combines the concepts of neurocontrol and intraspinal microstimulation. We hope with this circuit the locomotion of paralyzed hindlimbs of subjects, such as flexion, extension, in the future even standing and walking could be restored after complete spinal cord injury.

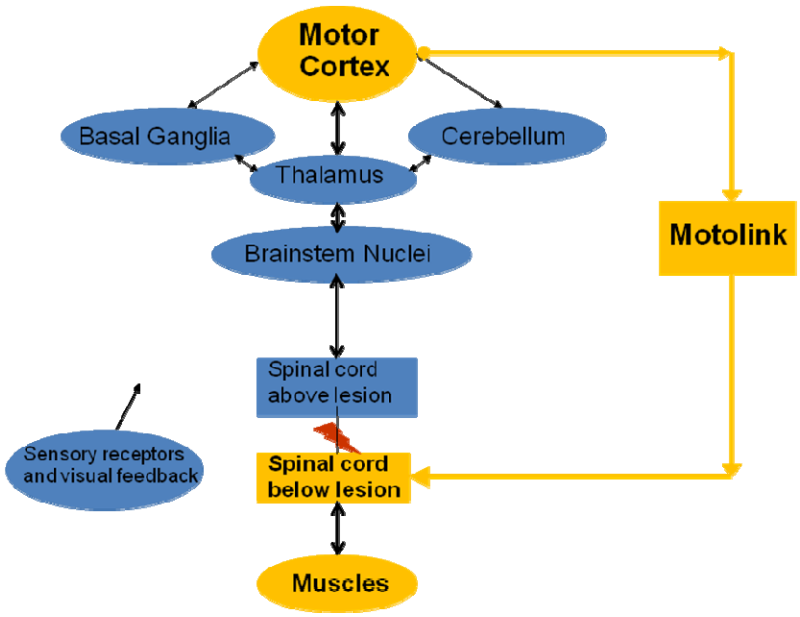

Figure 2.9 Artificial bypass of the corticospinal tract. In normal locomotion control, commands from the motor cortex control the spinal cord, and spinal cord innervate the muscles. In spinal cord injury, the cortical commands cannot activate the spinal cord below the lesion. In our design, Motolink reconnect the brain and the spinal cord by electrical circuit which is a bypass of the corticospinal tract.

2.5.3 Objectives of this study

To control motor neurons in the lumbar spinal cord by signals from the primary motor cortex using intraspinal microstimulation with self-made circuit, there are three objectives of this study to be achieved.

The first objective was to develop a research-designed circuit-Motolink, which contains an amplifier and a programmed stimulator. The amplifier is for signal acquiring and amplification. The stimulator is for stimulation generating and delivery. This part is based on engineering and computer science. It has been undergoing development by our technicians.

The second objective was to explore the effect of Motolink in bridging the brain and the spinal cord on acute experiments. We detected the neuron response in the visual cortex to visual stimuli, neuron response in the auditory cortex or medial geniculate body to auditory stimuli, to generate electrical stimulation. This stimulation activated the spinal motoneurons and elicited the muscles of the hindlimb on anesthetized rat. This bridging of the cerebral neurons and motoneurons in the spinal cord was very important for next steps.

The third objective was to explore the effect of Motolink in bridging the brain and the spinal cord on chronic animals. After spinal preparation chronically, guinea pigs were trained to walking on the treadmill by its forelimb. Signals from the hindlimb area of the motor cortex were recorded and converted to a pulse train of stimulation which was transmitted to ventral corn of the lumbar spinal cord. With “Motolink”, animals with paralyzed hindlimbs could show some recovery of hindlimb locomotor activity. With continue training on the treadmill with Motolink, the hindlimb movement of animals was expected to be improved gradually.

CHAPTER 3 NEURONAL ACTIVITIES FROM MEDIAL GENICULATE BODY, AUDITORY CORTEX AND VISUAL CORTEX WERE USED TO TRIGGER HINDLIMB MOVEMENT IN ANAESTHETIZED RATS

3.1 Introduction

This chapter describes the acute experiments conducted on rats. In the early stage of our project, we have developed a high-amplification electronic circuit for collecting extracellular signal from the brain and transforming the signal to stimulation which can innervate motoneuron in the spinal cord in anesthetized rats. Since spontaneous discharge and action potential of the motor cortex are suppressed by anaesthetic, we used neuronal activity from the visual cortex, auditory cortex, and medial geniculate body as a substitute to evaluate the effect of Motolink in using single neuronal activity from the brain to induce hindlimb movement in anaesthetized rats with transection in the spinal cord. This is the basement and premise of the chronic experiments in next chapter. The results of this chapter show the alternative sources of controlling signal of "Motolink" in the cerebrum. With reference to previous investigations of our lab, there are ON, OFF and ON-OFF auditory responses in the medial geniculate body of the guinea pigs and cats when noise-burst and pure-tone stimuli were applied to the ear contralateral to the recorded hemisphere (He 1997; He 2001; Guillery and Sherman 2002). These three types of MGB neurons were acquired in our study to trigger stimulation by Motolink.

Ten healthy adult rats with clean external ears were used for this experiment. In surgical preparation, the subjects were anaesthetized and received a transaction at the level of T12. Then craniotomy and rachiotomy were performed to expose the special

cortex and lumber spinal cord. Recording electrodes were implanted in the medial geniculate body, visual cortex and auditory cortex respectively, and stimulation electrode was implanted in the ventral horn of L2-L4 spinal cord. In recording section, the “Motolink” was connected to the recording and stimulating electrodes. Acoustic and visual stimuli were generated digitally by the TDT system. The interval of stimulus and duration of stimulus were changed accordingly. Extracellular recording was collected and delivered to remote computer for offline analyses by cables. Kinematics of the hindlimb movement was recorded by video simultaneously. At the end of each experiment, the subjects were sacrificed with overdose anesthesia and perfused with paraformaldehyde for the purpose of anatomical confirmation of the position of electrodes. Data were processed by software of AxoScope 10.2, Axon Clampfit 10.0, Adobe Photoshop 7.0 and SPSS 15.0.

3.2 Materials and Methods

3.2.1 Development of Motolink and microstimulator

A novel neuroprosthesis, named "Motolink", has been being developed in the Lab of Applied Neuroscience in the Hong Kong Polytechnic University. Motolink is a miniature prosthesis that amplifies neuronal signal from the brain and converts it into a train of pulses stimulation by a microprocessor (89C2051, ATMEL, San Jose, CA, USA). So far two versions of Motolink have been developed. The first version of Motolink has an amplifier and a stimulator, which needs cables to transmit the signals to remote computer, referred to Ver. 1.0 Motolink (Figure 3.1); the second version of Motolink has two amplifiers and one stimulator with both wired and wireless data transmission to remote computer, named Ver. 2.0 Motolink. In this chapter, we focus on the first version of Motolink which was used in acute experiments. The design of the second version is discussed in Chapter 4.

Ver. 1.0 Motolink was designed and developed in about one year. This Motolink contains an amplifier and a stimulator with common power supply from a battery. In order to separate the amplifier and the stimulator from mutual electronic interference, they are isolated by a transformer. There were four inputs in the amplifier, but in recording just one input was connected through switch manually by experimenter. There were seven outputs in the stimulator and only one output was set on through switch manually by tester when Motolink implemented stimulation. In this version, there were wires connecting between Motolink and remote computer for data storage and later analysis. The amplification of Motolink ranges from 1 to 10000 in theory. In practice, the maximum amplification is 4000 due to power consumption. Therefore the

amplification of Motolink is enough for both recording the electromyography signal of skeletal muscles in rats, the unit of which is millivolt; and for action potentials of the central nerve system, the unit of which is just microvolt. The stimulation is a train of 5 pulses with constant-current, 1 ms pulse width and 9 s interval between pulses. The frequency of stimulation is 20 Hz and amplitude is 3-7 V. The stimulation is set to be 3 ms delay of the trigger signal. In this experiment, Motolink acquired extracellular signals of the sensory cortex and MGB, amplified and converted them to a train of stimulation pulses.

To generate the visual stimuli, we developed a light-emission-diode which can generate white-light flash as vision stimulus. It has to be controlled by TDT system to be changed of the parameters of stimuli in program, such as the duration and the inter-stimulation interval. No figure is shown here.

Furthermore, to produce microstimulation in the spinal cord, a microstimulator has been developed for convenient identify the target segment of the spinal cord (Figure 3.2). The stimulation triggered is a train of 10 pulses in 100 Hz and of amplitude 6 V.

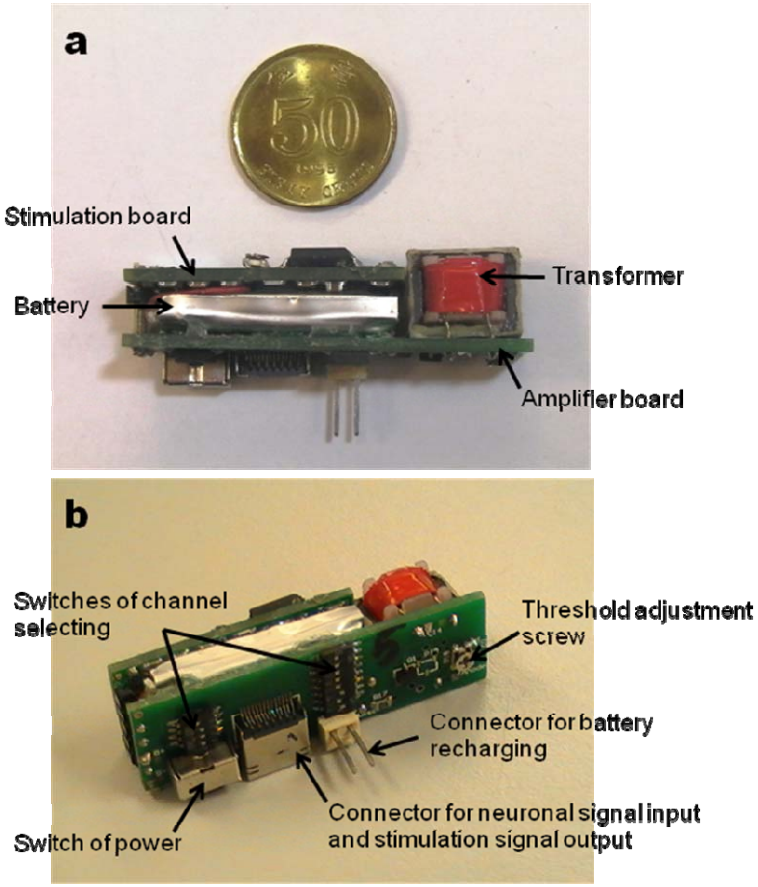

Figure 3.1 Ver. 1.0 Motolink. a. Ver. 1.0 Motolink included an amplifier board, a stimulator board and a battery. It was compared with a Hong Kong 50 cents coin. **b.** Lateral view of the amplifier of Ver. 1.0 Motolink. There were a multichannel connector for signal input and output, a connector for battery recharging, a threshold adjustment screw and several switches.

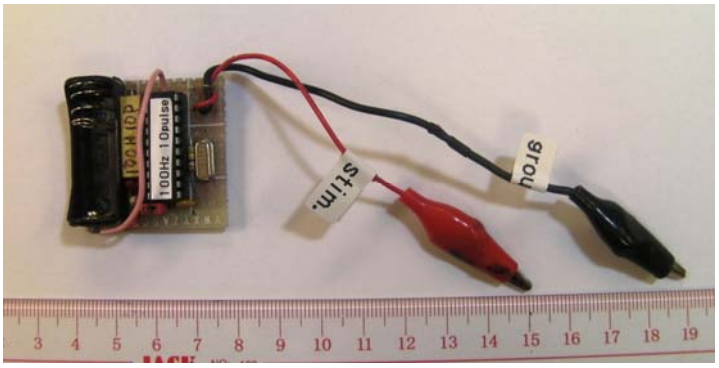

Figure 3.2 Microstimulator. It is designed for generating microstimulation to identify the spinal segment in acute experiments.

3.2.2 Surgical procedure

3.2.2.1 Subjects

1. Ten healthy adult sprague dawley rats (220-380g, either sex, SPF) with clean external ears and bright eyes were used as subjects. Before experiment, the rats were kept in the Laboratory Animal Center at constant temperature (22ºC) and on a regular 12-hour light/dark cycle. All experiments were conducted in compliance with the *Principles of Laboratory Animal Care* (National Institutes of Health, No. 86-23, revised 1985) and all experimental procedures were approved by the Animal Subjects Ethics Sub-Committee of the Hong Kong Polytechnic University. The experimenters have gotten the Personal License of Animal Regulations approved by the Department of Health in Hong Kong.

   Subject was mounted in a stereotaxic device in a double-walled soundproofed room (NAP, Clayton, Australia). Ketamine hydrochloride (35 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) with the maintaining dose of 5 mg/kg ketamine and 2 mg/kg xylazine per hour were administered through the procedure of surgery. In the acute experiment, anaesthetized animals were maintained for about 12 hours. Animal's body temperature was controlled between 37.5 and 38.5 °C by homoeothermic monitoring system.

Figure 3.3 Adult sprague dawley rat was used as subjects.

3.2.2.2 Electrode implantation of extracellular recording

Craniotomy was performed to enable electrodes access the auditory cortex, visual cortex, and medial geniculate body respectively. Single tungsten electrode with high-resistance (2-4 MΩ, FHC Inc., Bowdoin, ME) was transiently implanted in the MGB, AC and VC for recording the activities of neurons, and its reference electrode was placed on the cortex near the Bregma. The gradual accessing of electrode was controlled by a stepping-motor micro-manipulator system outside the sound-proof room.

3.2.2.3 Electrode implantation of intraspinal microstimulation

Subjects received a transaction at the level of T12 to serve as a model of spinal cord injury. Rachiotomy was performed to expose the spinal cord for introspinal microstimulation. According to the mapping of thoracic and lumbar spinal cord, single tungsten electrode with low-resistance (10-30 KΩ, FHC Inc., Bowdoin, ME) was placed in the right L2-L4 spinal cord which activates right hindlimb movements, and its reference electrode was placed in the erector spinae muscles nearby.

3.2.3 Mapping of thoracic and lumbar spinal cord

To determine the relative arrangement of locomotor recruitment pool in the thoracic and lumbar spinal cord in rats, coarse mapping was investigated in T7-L6 segments of spinal cord. This was an acute study and each experiment lasted for about 8 hours.

Five healthy Sprague-Dawley rats (220-380g, female) were applied as subjects. Before experiment, the rats were kept in the Laboratory Animal Center at constant temperature (22ºC) and on a regular 12-hour light/dark cycle. Experimental procedure was approved by the Animal Subjects Ethics Sub-Committee of the Hong Kong Polytechnic University.

Subject was mounted in a stereotaxic device in a double-walled soundproofed room (NAP, Clayton, Australia). Ketamine hydrochloride (35 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) with the maintaining dose of 5 mg/kg ketamine and 2 mg/kg xylazine were administered through the procedure of surgery. Animal's body temperature was controlled between 37.5 and 38.5 °C by homoeothermic monitoring system.

Rachiotomy was performed to expose the spinal cord for locomotor mapping. Signal generator together with current isolator (ISP, USA) generated the stimulation of square-wave current with low frequency (70-150 Hz), short duration (0.5-1 ms) and small current (40-100 μA, (Mushahwar and Horch 2000). The stimulation was delivered to the thoracic and lumber spinal cord through low resistance tungsten electrode (10-50 KΩ, FHC Inc., Bowdoin, ME). Each stimulating site was distributed with 0.5 mm intervals from anterior to posterior in the ventral horn of the right spinal

cord. Reference electrode was placed on the muscles near the low back. If there were response of body part to the stimulation, the responsive body parts and the location of stimulation were recorded immediately.

At the end of experiments, subjects were sacrificed by overdose of pentobarbital sodium (60 mg/kg, i.p.).

3.2.4 Acoustic and visual stimulation protocol

3.2.4.1 Acoustic stimulation protocol

In the recording section, acoustic stimuli were generated digitally by TDT system 3 (Tucker-Davis Technologies, Alachua, FL). Repeated noise bursts with frequencies ranging from 100 Hz to 35 kHz were delivered through a microphone to the left ear of rat via a hollow ear bar. Inter-stimulus interval (ISI) of auditory stimuli was changed from 1 s to 10 s, and duration of the stimulus was changed from 100 ms to 1000 ms.

3.2.4.2 Visual stimulation protocol

Visual stimuli were generated through a self-manufactured light-emission-diode. The light-emission-diode was controlled by the TDT workstation. In the recording section, it was placed to face the left eye of subject. Repeated white-light flash was employed. Inter-stimulus interval of visual stimuli was changed from 1 s to 10 s, and duration of the stimulus was changed from 100 ms to 500 ms.

3.2.5 Histology

At the end of the study, rats were deeply anesthetized with overdose of pentobarbital sodium (60 mg/kg, i.p.; Sigma) and were perfused transcardially with 400 ml 0.9% NaCl, followed by 400 ml ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brain tissue was quickly removed from the cranial bones. After post-fixation by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4), and dehydration by 30% sucrose in 0.1 M phosphate buffer (PB, pH 7.4), frozen section was conducted. The sagittal sections of spinal cord were sliced in 40 μm which containing the transected site. After complete dryness, Nissl staining was performed to these sections. Photograph of these sections with Nissl staining was taken for anatomical confirmation of the lesion of transection.

3.2.6 Data recording and analysis

After surgical preparation, Motolink was connected to both recording electrode and stimulating electrode. When we gave stimuli to subject and turned on the Motolink, the extracelluar signals were delivered through connector to remote computer. These data was A/D-converted (Axon Digidata 1440, Molecular Devices Co., Chicago, IL) and filtered (sampling 10000 Hz, band pass 300 Hz-3 kHz) before stored in the computer together with the artifact of auditory and visual stimulus signals. The effect of Motolink leading movement of hindlimb was monitored by video (Resolution 720×576，GZ-MG27AH, JVC, Japan) in real time.

Extracelluar data were recorded by AxoScope 10.2 (Molecular Devices Co., Chicago, IL) and processed by Axon Clampfit 10.0 (Molecular Devices Co., Chicago, IL). Adobe Photoshop 7.0 and SPSS 15.0 were used for data processing and statistics analysis. T-test was used for comparison of time lag between hindlimb and forelimb at different speed.

3.3 Results

3.3.1 Comparison of EMG recording between the TDT system and the amplifier of Motolink

At first, to test the performance of Motolink, we compared the electromyography signal recorded by TDT system and Motolink. The TDT system is commercial workstations which integrate data acquisition, stimulus presentation, and analysis for neurophysiology, evoked potentials, and psychophysics. We found that the EMG activity recorded by amplifier is closely similar to the signals recorded by TDT system, except for the reverse phase and some high frequency noise in the recording of Motolink (**Figure 3.4**). These are due to the three-grade amplification of Motolink.

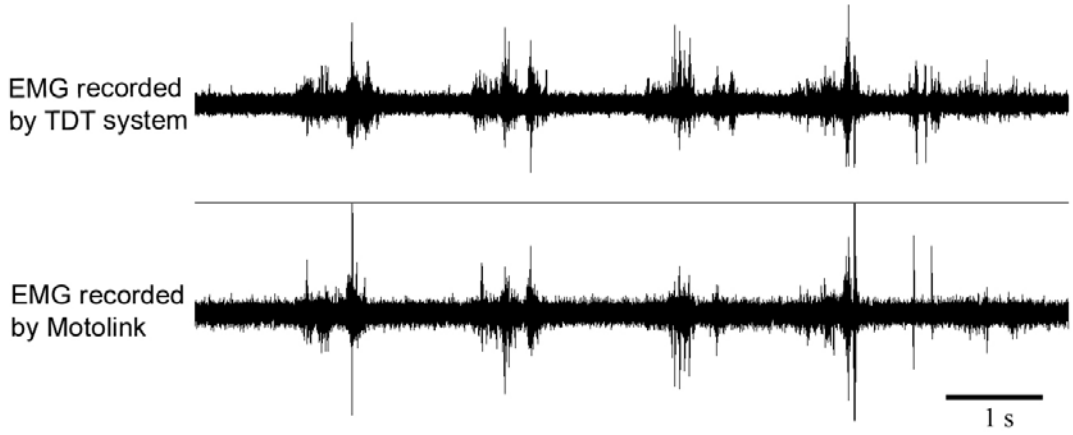

Figure 3.4 Comparison of electromyography signal recorded by TDT system and Motolink. Upper panel: EMG recorded by TDT system. Lower panel: EMG recorded by Motolink.

3.3.2 Mapping of thoracic and lumbar spinal cord

Three rats were investigated in the experiments of mapping partial thoracic and lumbar spinal cord. The spinal cord was targeted at the ventral horn of the spinal cord between T7 to L6 segments in the right side. Table 3.1 shows different body parts were innervated by electrical stimulation. The rostral-caudal organization of motor pool in the spinal cord was found to be corresponded well with anatomy of the body. When stimulated with a current of 40 -100 μA, we observed that L1-L2 spinal cord contains the motor pool of the hip and knee, L3 spinal cord includes the representation of the ankle, L4 spinal cord comprises motor neurons of distal hind leg and L5 spinal cord can be activated with tail movement. Therefore, the lumbar spinal cord mainly includes the motor pools of the hindlimb. Motor neurons which control the muscles of abdomen and lower back were located in the lower thoracic spinal cord, besides the segments of T12 and T13 control movement of hindlimb as well.

Table 3.1 Representation of the lower thoracic and lumbar spinal cord

Level of the Spinal Cord	Body parts responded to stimulation
T7	Lower back and abdomen
T8	Lower back and abdomen
T9	Lower back
T10	Lower back
T11	Lower back
T12	Lower back and hip of hindlimb
T13	Lower back and hip of hindlimb
L1	Hip and knee of hindlimb
L2	Hip and knee of hindlimb
L3	Ankle of hindlimb
L4	Distal hindlimb
L5	Tail
L6	Tail

3.3.3 Neuronal response of medial geniculate body was used to produce hindlimb movement

Seven neurons of the auditory thalamus have been recorded acutely in this study. Three are OFF neurons, two are ON neurons and two are ON-OFF neurons of MGB. Their auditory responsive and triggering stimulation were sampled in Figure 3.5 , Figure 3.6 and Figure 3.7 respectively.

3.3.3.1 Neural response of OFF neuron of MGB was used to trigger stimulation and produce hindlimb movement

The neuron in Figure 3.5 was an OFF neuron in MGB that responded to repeated auditory stimuli with high-amplitude spikes (Figure 3.5 b-c, stimulator was turned off). The response occurred in synchrony with the offset of the stimulus when the duration was lengthened (Figure 3.5 c). Neuronal spikes were detected at a threshold of 0.7 V and triggered the generation of pulse trains in the microprocessor. Pulse trains were delivered through a bipolar electrode to spinal motor neurons. Pulses in the SC produced an electrical artifact at the recording electrode in return. Figure 3.5 d shows representative neuronal signals captured by the recording electrode and the artifact generated by the pulse trains stimulation of the SC. A train of five pulses was triggered by the OFF response (Figure 3.5 d, left panel). Artifact caused by the electrical stimulation of each pulse in the train is shown in the right panel of Figure 3.5 d. The triggering spike was detectable at the beginning of first pulse, as indicated by the arrow. The amplitude matched the threshold set by the experimenter. Electrical stimulation was responsive to the OFF response for 140 s (Figure 3.5 e). Upon 33

repeats of the stimulus, LMNs were triggered 35 times (31 were triggered by OFF responses, 4 by spontaneous responses of the MGB neuron).

The electrical stimulation of LMNs in the spinal cord caused movement of the right hind limb (see synchronized video referenced to Figure 3.5 e in the attachment CD disk, Movie 3-1, Figure 3.5 e_Movie). The noise bursts on the sound tracks of the video was the acoustic stimuli delivered to the left ear of the subject.

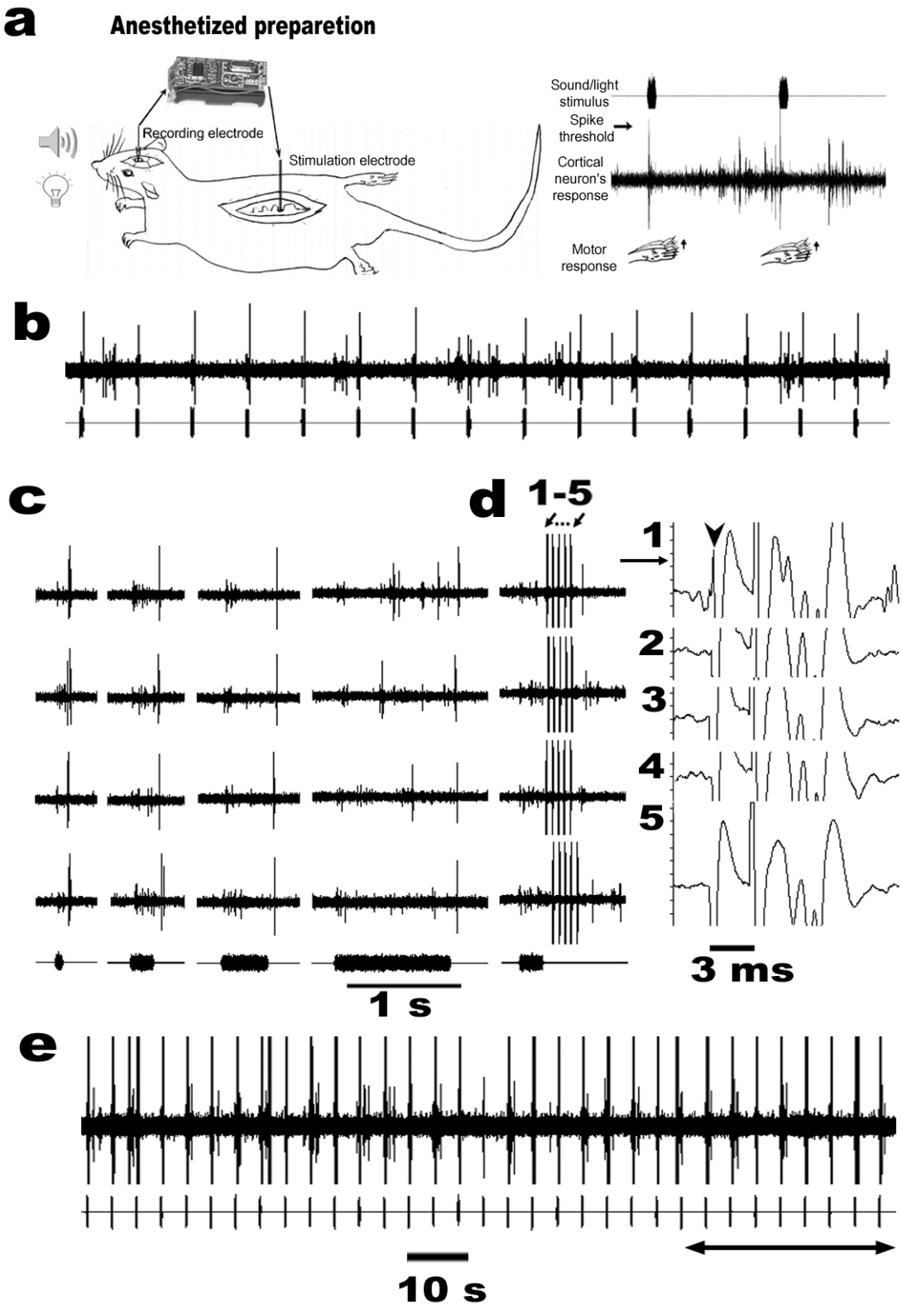
a
Anesthetized preparetion
Recording electrode
Stimulation electrode
Sound/light stimulus
Spike threshold
Cortical neuron's response
Motor response
b
c
d
1-5
1
2
3
4
5
3 ms
1 s
e
10 s

Figure 3.5 OFF response in the MGB triggered electrical stimulation in the spinal cord which produced hind limb movement. a. Schematics of Motolink in connecting the brain and the spinal cord on anesthetized rats. In the left panel, Motolink links the brain and the spinal cord via recording electrodes in the brain and stimulation electrodes in the spinal cord. In the right panel, the cortical neuronal response of sound and light stimuli triggers twitch movement of the hindlimb. **b.** Extracellular recording of neuronal responses to repeated stimuli. **c.** An OFF response neuron, which responded with spikes at stimulus offset. Stimulus duration was changed from 90 ms to 1 s. **d.** Left panel: Recording of an MGB neuron when an acoustic stimulus was delivered to the subject. Its auditory response was used to trigger electrical shocks in the spinal cord. When the electrical shocks of 5 pulses (3-ms mono-polar, inter-stimulus interval: 20 ms, 5 V) was applied to the spinal cord, artifact was picked up at the recording electrode as marked by the oblique arrows. Right panel: Artifact of each pulse depicted from a pulse train in the left panel as marked by 1-5. A spike was distinguishable before the artifact of the first pulse (Arrow head). These conventions apply to the following figures. Since no time delay between the detection of spikes and application of shocks was set in this experiment, a spike with amplitude of 0.7 V (threshold for this neuron marked with horizontal arrow) always preceding the artifact of the first pulse, but not the following four pulses in the train. e. A recording of 140 s from the recording electrode in the auditory thalamus. Artifacts were caused by electrical shocks in the spinal cord. Part of the synchronized video movie, with its duration marked with a double-arrow line is attached in the supporting materials (Movie 3-1, Figure 3.5 d_Movie).

3.3.3.2 Neural response of ON neuron of MGB was used to trigger stimulation and produce hindlimb movement

ON type neuron was found in MGV as Figure 3.6 shown. The response of this neuron is with high amplitude spikes and synchronized with the onset of stimuli. It was observed that the response to the onset of stimuli changed correspondently when the duration of stimuli was extended (Figure 3.5 b). The ON response to auditory stimuli triggered 5 pulses train when the amplified signal was delivered to the microprocessor and the output of pulses trains were transmitted to the stimulation electrode in the spinal cord as the OFF neuron did. The action potential of MGB neurons were detected up to the threshold of 0.3 V. The number of stimulation train/trains depended on the duration of the stimulus. More than one train was triggered by the ON response in the MGB when the duration was extended to 1 s (right panel of Figure 3.6 d). Artifact of each pulse in the 5 pulse train is shown in the Figure 3.6 c and d. The electrical stimulation was quite reflective to the ON response in a period of 185 s (Figure 3.6 c). The electrical stimulation of the motor neurons in the spinal cord was observed to cause movement of the right hind limb. During this period with 73 repeats of the stimuli, the motor neurons in the spinal cord were triggered for 89 times, in which 73 were triggered by ON responses and 16 by spontaneous responses of the MGB neuron.

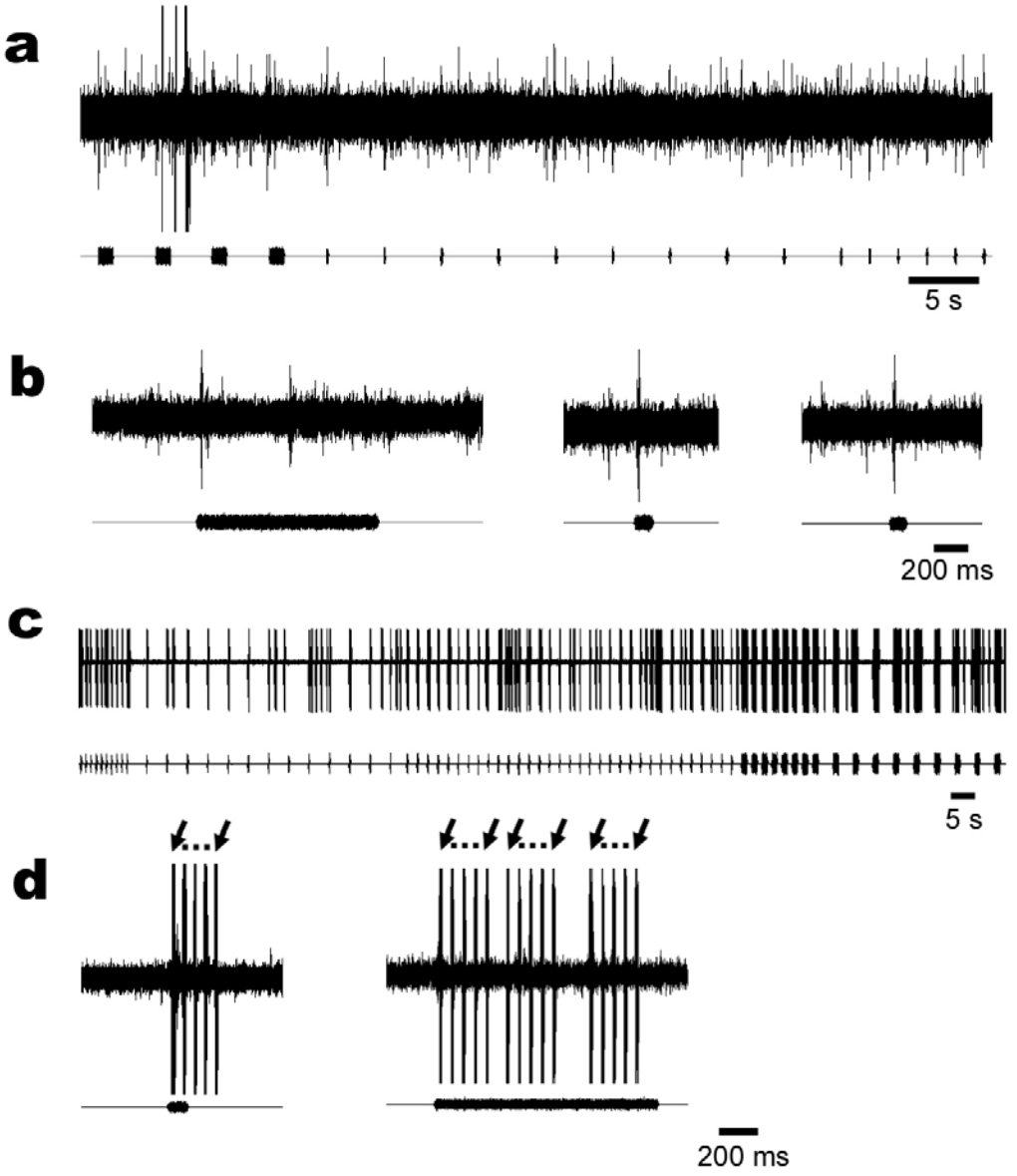

Figure 3.6 ON response in the MGB triggered electrical stimulation in the spinal cord which produced hind limb movement. a. Extracellular recording of neuronal responses to repeated auditory stimuli in a period of 66 s. **b.** ON neuron with spikes response at the stimulus onset. It responded to acoustic stimuli correspondingly as the duration and ISI of stimuli changed. The density and number of stimulation train triggered by Motolink are corresponding to the ISI and duration of the acoustic stimuli. **c.** Recording of this neuron in 185 seconds. Artifacts were caused by electrical shocks in the spinal cord. **d.** Left panel: one train of stimulation was generated by the response of ON neuron to 100 ms stimulus. Artifact was picked up at the recording electrode as marked by the arrow heads; Right panel: three train of stimulation was generated by the response to 1 s stimulus.

3.3.3.3 Neural response of ON-OFF neuron of MGB was used to trigger stimulation and produce hindlimb movement

We also recorded an ON-OFF type neuron which's response is synchronized with both the onset and offset of stimuli. It was recorded that the response to the onset and offset of stimuli changed correspondently when the duration of stimuli was extended (Figure 3.7 b). When the threshold was set on a high level, we found only the response to the offset of auditory stimuli triggered a train of stimulation pulses (Figure 3.7 d). The pulses trains were transmitted to the stimulation electrode in the spinal cord as the OFF and ON neuron did. The action potential of MGB neurons were detected up to the threshold of 0.7 V. Artifact of each pulse in the 5 pulse train is also observed. The electrical stimulation was quite reflective to the ON-OFF response in a period of 54 s (Figure 3.7 c). Electrical stimulation of the motor neurons in the spinal cord produced movement of the right hind limb. During this period with 14 repeats of the stimuli, the motor neurons in the spinal cord were triggered for 15 times, in which 13 were triggered by ON responses and 2 by spontaneous responses of the MGB neuron, and 1 response to stimulus did not trigger any stimulation because it was below the threshold.

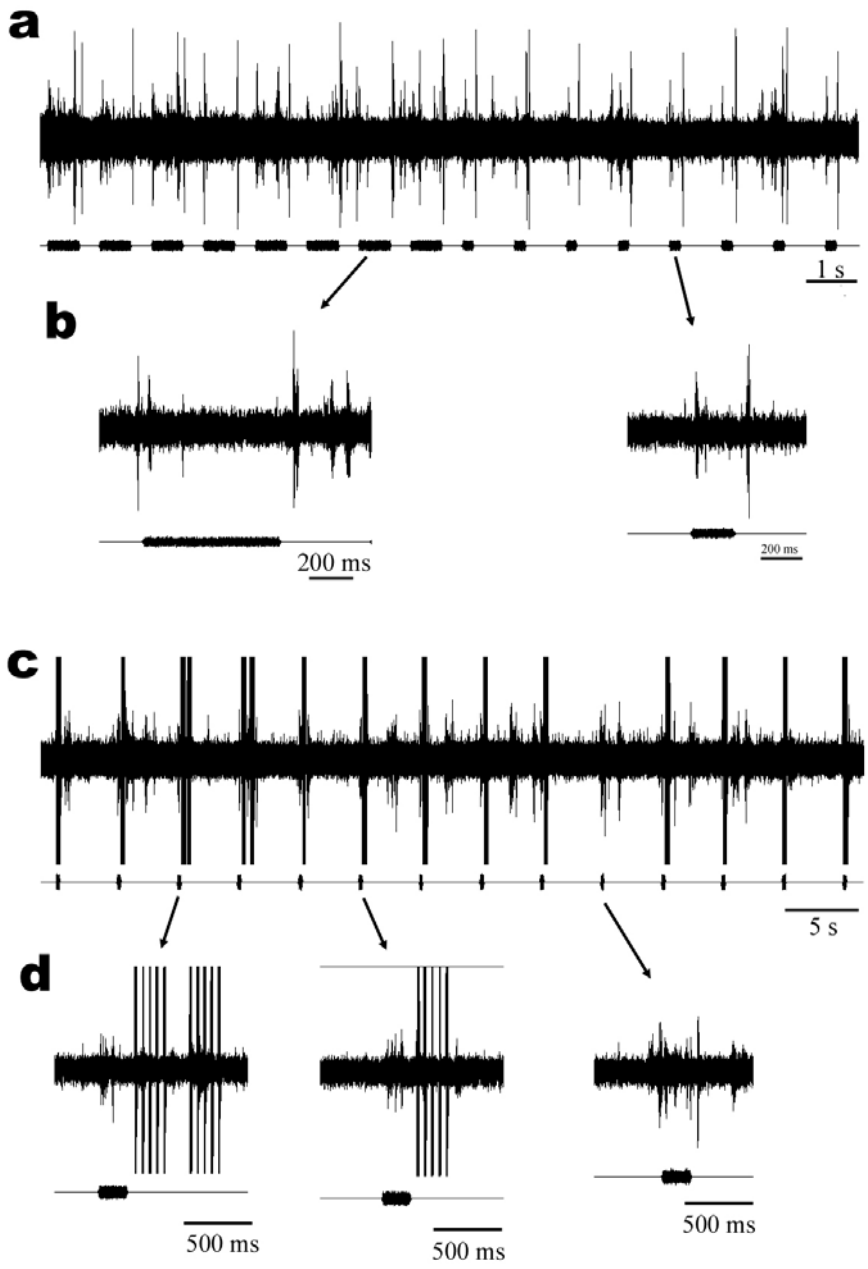

Figure 3.7 ON-OFF response in the MGB triggered electrical stimulation in the spinal cord which produced hind limb movement. a. Extracellular recording of neuronal responses to repeated auditory stimuli in a period of 16 s. **b.** The neuron was an ON-OFF neuron which responded with spikes at the onset and offset of the stimulus. It responded to acoustic stimuli correspondingly as the duration and ISI of stimuli changed. **c.** Recording of this neuron in 54 seconds. Artifacts were caused by electrical shocks in the spinal cord. **d.** Left panel: one train of stimulation was generated by the response to 100 ms stimulus, which was followed by another train of stimulation triggered by spontaneous activity; Middle panel: one train of stimulation was generated by the response to 100 ms stimulus; Right panel: no stimulation was triggered.

3.3.4 Neural response of auditory cortex was used to trigger stimulation and produce hindlimb movement

In order to examine the pulse stimulation triggered by cortical neuron response to the auditory stimuli, neuronal activity of auditory cortex was studied. Eight ON type neurons of the auditory cortex responded to the auditory stimulus with spikes at the onset (a representative neuron is shown in Figure 3.8 a). Neuronal responses to a long-lasting repeated acoustic stimulus, with different inter-stimulus intervals, triggered reliable electrical stimulation of the SC. (Figure 3.8 d). With a duration of 90 ms, the microprocessor generated one train (5 pulses) for each stimulus, while it generated two or four pulse trains (10 or 20 pulses) for each stimulus when the stimulus duration was lengthened to 270 ms (Figure 3.8 b-c). The stimulation electrode was placed at the level of L5. The stimulation electrode was placed at the level of L5. It triggered a movement of digit 2 of the right hindlimb (Synchronized video of the digit movement, see Movie 3-2, Figure 3.8 d_Movie, 67 s). During the period with 98 repeats of the stimulus, the motor neurons were triggered for 104 times (from the onset of stimulus, the multiple trains were counted as once) with which 96 were triggered by auditory responses and 8 by spontaneous responses of the AC neuron. The SC was partially sectioned at the level of thoracic vertebrae T12. Nissl staining shows the extent of a representative SC lesion in Figure 3.8 e.

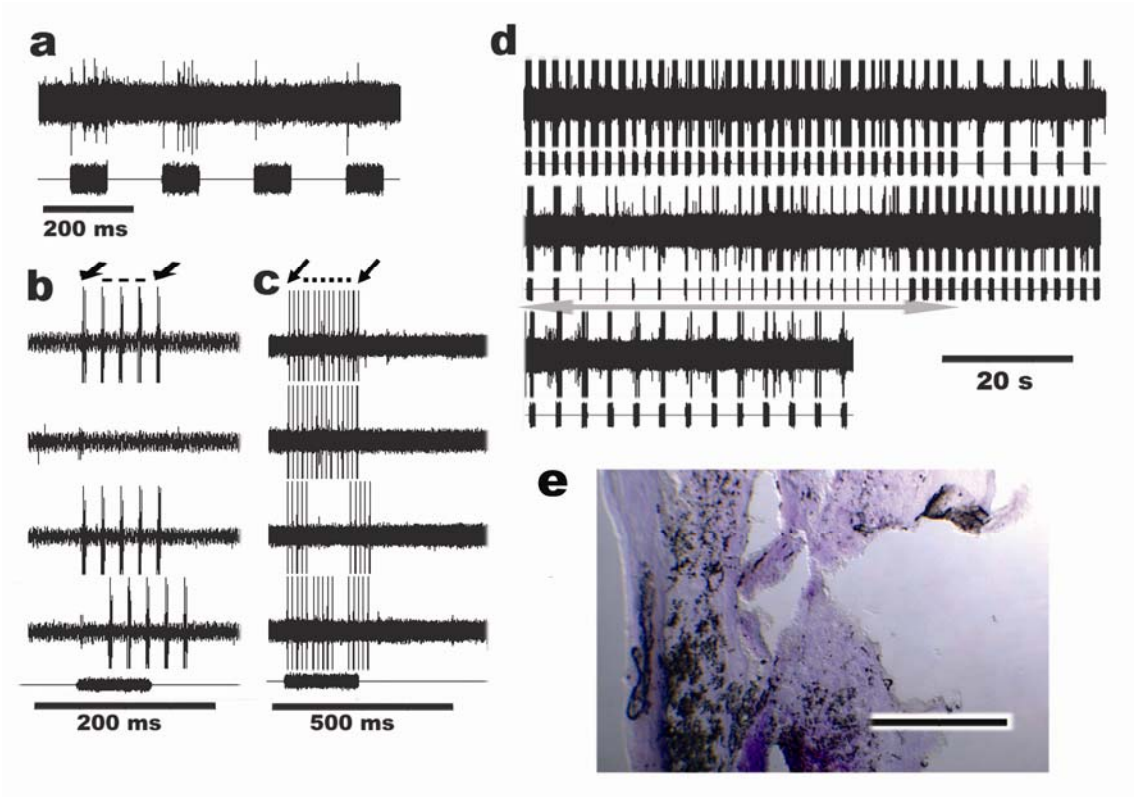

Figure 3.8 Auditory response in the cortex triggered electrical shocks in the spinal cord which caused digit movement. a. Extracellular recording of neuronal responses to repeated stimuli. **b-c**. Recording at the cortical neuron when acoustic stimulus was delivered to the animal and its auditory response was used to trigger electrical shocks in the spinal cord. Artifact of electrical stimulation was picked up at the recording electrode as marked by the arrow heads. Stimulus of a short duration (90 ms) triggered a train of five pulses, or none on a few occasions (**b**), while a longer duration triggered 2-3 trains of five pulses (**c**). **d.** A recording of 232 s when the auditory response triggered electrical shocks in the spinal cord. Part of the synchronized video movie, marked with a double-arrow line, is attached as Movie 3-2 (Figure 3.8 c_Movie, 67 s). **e**. Nissl staining shows the sectioned spinal cord at thoracic vertebrae T12. Scale bars: 0.5 mm.

3.3.5 Neural response of visual cortex was used to trigger stimulation and produce hindlimb movement

Furthermore, seven neurons from the visual cortex were recorded. Signals from these neurons were fed to a microprocessor, which then delivered pulse trains to the SC. Figure 3.9 shows a representative response of a VC neuron to a light stimulus. Neuronal responses to light stimuli of different durations and ISIs triggered electrical pulses to the SC (Figure 3.9 b-c). Electrical stimulation of motor neurons in the SC caused movement of the right hindlimb (see Movie 3-3, Figure 3.9 c_Movie, 35 s, which is synchronized to the double arrow marked period of Figure 3.9 c). Repeated white-light flashes were captured by the video camera. Muscle fatigue was observed near the end of the video clip, after sustained exposure to a quickly-repeating stimulus. When the stimulus was repeated 214 times, motor neurons of the spinal cord were triggered 194 times. Visual stimuli triggered 192 of these responses, while two were triggered by spontaneous responses of the VC neuron.

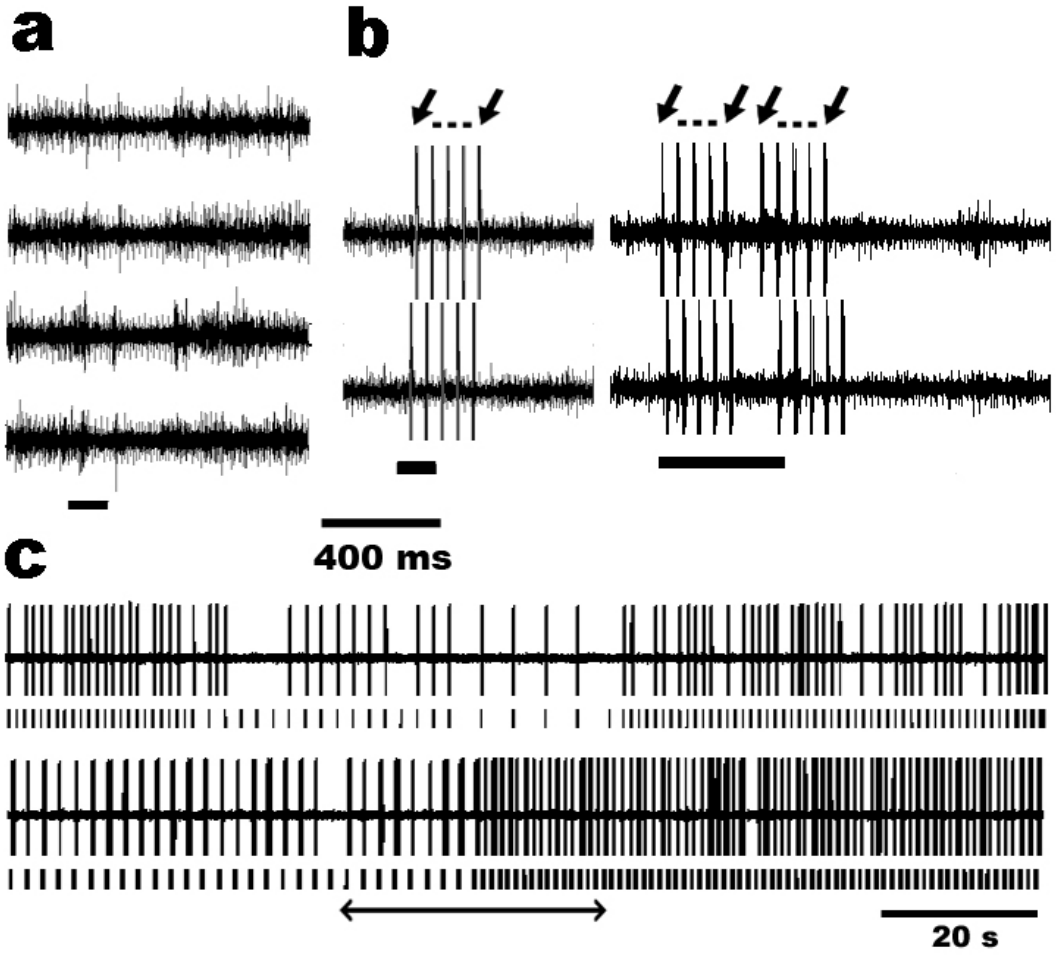

Figure 3.9 Visual response in the cortex triggered electrical shocks in the spinal cord which caused hindlimb movement. a. Extracellular recording of neuronal responses to repeated stimuli. The horizontal bar indicates the right stimulus. **b**. Recording at the cortical neuron when light stimulus was delivered to the subject and its visual response was used to trigger electrical shocks in the spinal cord. Artifact of electrical stimulation was picked up at the recording electrode, as marked by the oblique arrow heads. Stimulus of a short duration (90 ms) triggered a train of five pulses, while a longer duration triggered two trains of five pulses (right panel). **c**. A recording of 262 s, when the visual responses triggered electrical shocks in the spinal cord. A movie synchronized to a period marked by double-arrow is attached in Movie 3-3 (Figure 3.9 c_Movie, 35 s).

3.4 Discussion

We have developed a low-noise, high-gain amplifier that can successfully read neuronal signals of the brain. The amplifier is connected to a programmed microprocessor that generates a pulse train to directly activate SC motor neurons, which, in turn, activate muscles. Before the circuit was tested in awake, behaving animals, we evaluated it in anesthetized animals. Neuronal responses to sensory stimuli in the auditory thalamus, auditory cortex and visual cortex were used to trigger stimulation of LMNs that control movement of the hindlimb. There were two main reasons why we did not directly read UMN signals in anesthetized animals: 1) it was difficult to determine that the signal was a motor signal, and 2) motor neurons do not show much spontaneous activity under anesthesia.

3.4.1 Rat auditory and visual responses

We recorded eight neurons from auditory cortex, seven neurons from the MGB and seven neurons from visual cortex. The neuron shown in Figure 1 was an OFF neuron likely located at the non-lemniscal MGB whose response followed the offset of the stimulus when the stimulus duration was lengthened (He 1997; He 2001; Guillery and Sherman 2002; He 2003; He 2003). Neurons in the AC (Figure 3.8) and VC (Figure 3.9) showed more spikes when the stimulus duration was lengthened; these neurons could be categorized as long-duration selective neurons (He, Hashikawa et al. 1997).

3.4.2 Stimulation of spinal motor neurons

For stimulation, we used monopolar pulse trains of five pulses (20 Hz, 3 ms). Instead of using a constant current, we used a constant voltage of 3 – 6 V. Since the impedance of the stimulation electrode was 50-100 kΩ, the stimulation current was between 30 – 120 μA, which is comparable to the intraspinal stimulation current used in other investigations (Saigal, Renzi et al. 2004; Mavoori, Jackson et al. 2005) and our previous cortical stimulation studies (He, Yu et al. 2002; Xiong, Yu et al. 2004; Yu, Xiong et al. 2004). We used five pulses (20 Hz/pulse train) to enable us to distinguish the twitching movement of the limbs and tail from natural body movements.

Intraspinal microstimulation, with currents ranging from 1-4x threshold (mean threshold 32 μA), could generate bilateral, phasic stimulation-induced walking in cats (Mushahwar, Collins et al. 2000; Mushahwar, Gillard et al. 2002; Saigal, Renzi et al. 2004). Motor neurons in the cat SC are topographically localized (Mushahwar and Horch 2000; Mushahwar and Horch 2000). In the present study, we selected different site to activate LMNs of anesthetized rats. As shown in the video clips, we could selectively activate the movement of a single digit with the extracellular stimulus.

3.5 Summary

The above findings indicated that the amplifier and stimulator of Motolink could successfully acquire the neuronal signals in the MGB, AC and VC to related stimuli, and the programmed microprocessor could generate the pulse train to activate the lower motor neurons in the spinal cord, which innervate the movement of the hind limbs. A miniaturized circuit would enable us to re-bridge the brain and the dissected spinal cord through single-channel.

However, the study of this chapter cannot fulfill the goal of reconnect the upper motor center and the lower motor center. Therefore, the next step of this study was to relay the motor neurons in the primary motor cortex to those in the spinal cord in conscious rats or guinea pigs with spinal cord injury.

CHAPTER 4 NENRONAL SIGNAL FROM THE PRIMARY MOTOR CORTEX WAS USED TO TRIGGER HINDLIMB MOVEMENT IN BEHAVING GUINEA PIGS WITH SPINAL CORD INJURY

4.1 Introduction

Since the ability of Motolink in reconnection of cerebral neurons and lower motoneurons in acute experiments has been proved efficiently, we wondered whether upper motor neurons can be reconnected with the lower motor neurons in behaving animals with spinal cord injury. To pursue this goal, neural activities of the hindlimb area of primary motor cortex were used to trigger stimulation which caused hindlimb movement during treadmill walking. Chronic experiments have been conducted on guinea pigs with spinal cord injury. Subjects were supported by harness, which made it possible to walk on the treadmill by its forelimbs (Figure 4.1).

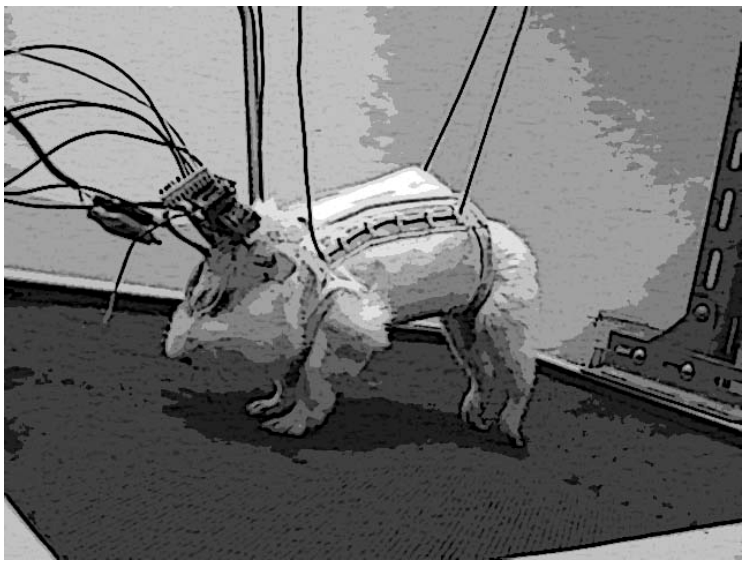

Figure 4.1 Guinea pig as spinal cord injury model was walking on the treadmill with Ver. 1.0 Motolink. The circuit is Motolink, which is holding on guinea pig's head. Wires link to computer for signal storage and off-line analysis.

For the purpose of using neural activity of the motor cortex, especially of the hindlimb region, the motor representations of the hindlimb area in the primary motor cortex has been identified at first. Mapping of the right side prefrontal neocortex in guinea pigs was performed using intracortical microstimulation methods. After confirmation of that, we conducted surgery on guinea pig to implant recording electrodes into the right-side hindlimb area of M1, stimulating electrodes into the lumbar spinal cord, and recording electrodes of EMG into the muscles of left forelimb. Three kind of cortical electrode arrays were applied. We recorded data of neural activity, EMG signals and kinematics after several days of recovery. Data was transferred to remote computer by wire or wireless at different stage.

4.2 Materials and Methods

4.2.1 Design of Motolink with wire or wireless transmission to computer

4.2.1.1 The Ver. 1.0 Motolink was used at early stage

Before development of the second version of Motolink, we used the first version together with a two-amplifier circuit to record chronic data, in which the signal was delivered to remote computer through cables (Figure 4.2 c). The two-amplifier has been designed for recording the activity of electromyography (Figure 4.2 b). The schematic of this two amplifiers board is identical to that of the amplifier on the first version Motolink. It is 7 gram and less than two times length of a Hong Kong 50 cents coin. After combined with the two-amplifier, the total weight of Ver. 1.0 Motolink is 22 gram.

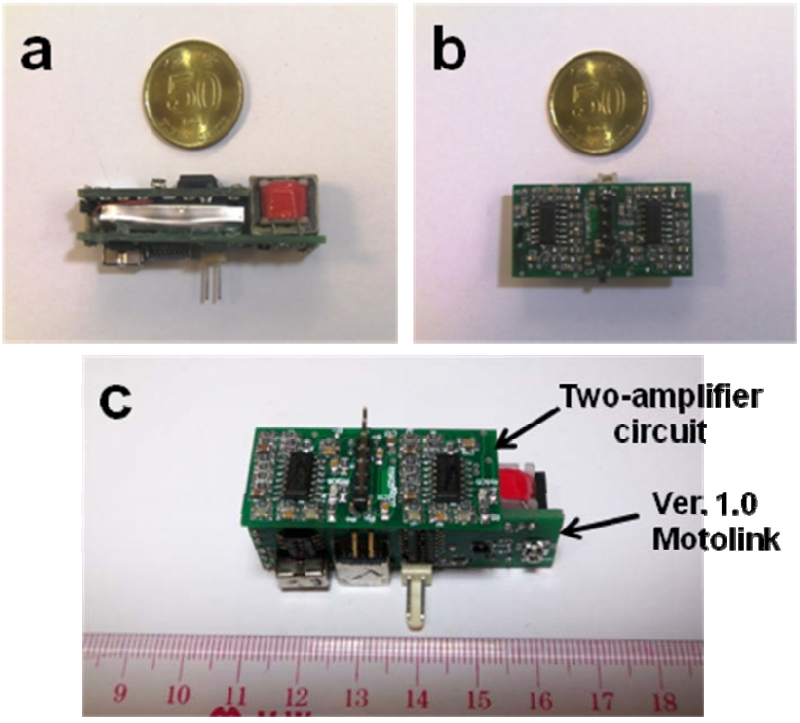

Figure 4.2 The first version of Motolink with attached amplifier circuit. a. Ver. 1.0 Motolink is compared with a Hong Kong 50 cents coin. **b.** Two-amplifier circuit is compared with a Hong Kong 50 cents coin. There are two amplifiers in the circuit, but we just used one channel as recording. **c.** To record neuronal activity and EMG spontaneously, two-amplifier board is attached to Ver. 1.0 Motolink. The whole circuit was held on the head of guinea pig when in recording.

4.2.1.2 The Ver. 2.0 Motolink has been developed at later stage

Telemetry is a technology that allows wireless data transfer and remote measurement. In this study, it refers to data report to computer wirelessly. This function is achieved by Ver. 2.0 Motolink. After the second version has been developed, wireless transmission was conducted to report extracellular signals to external computer. The second version of Motolink has been in developing in more than two years because of a large number of technical issues needed to be solved, such as the requirement of a lighter and smaller design, together with telemetry technique. As mentioned in Chapter 3, the second version of Motolink has two amplifiers (8 g)

and one stimulator (6 g) altogether (Figure 4.3 a). The size of the amplifier board is 50*22 mm and the stimulator board is 32*22 mm. This version could transmit signals to external computer either by cables or wirelessly (Figure 4.3 b). It is for application in conscious guinea pigs with spinal cord injury. The telemetry technique is implemented by radio frequency (RF) transmitter (Figure 4.3 c) and frequency modulation (FM) receiver (Figure 4.4). The RF of transmitter ranges from 88.0 Hz to 99.0 Hz.

The additional amplifier is designed for electromyography recording, which is regarded as signal reference to cortical signals from the hindlimb area of M1. Three parts are independently supplied with three batteries, which isolate them from electronic influence mutually. An optocoupler is applied for the signal transmission between the amplifier and stimulator, which isolates two parts from electronic interference.

There were four inputs in the amplifier, but only one input was turned on manually by researcher.during recording And there are five outputs in the stimulator, while only one output was set on by tester when Motolink implemented stimulation. The range of amplification of Motolink is from 1 to 10000 in theory; and in practice, the maximum amplification is 4000, which is the same as the first version.

The figure of Ver. 2.0 Motolink is smaller and thinner than that of Ver. 1.0 Motolink. Two versions are compared with a 50 cents coin in Hong Kong (Figure 4.5). The weight of the second version set is only 14 gram which is easily for animal to carry on than that of Ver. 1.0 (22 g).

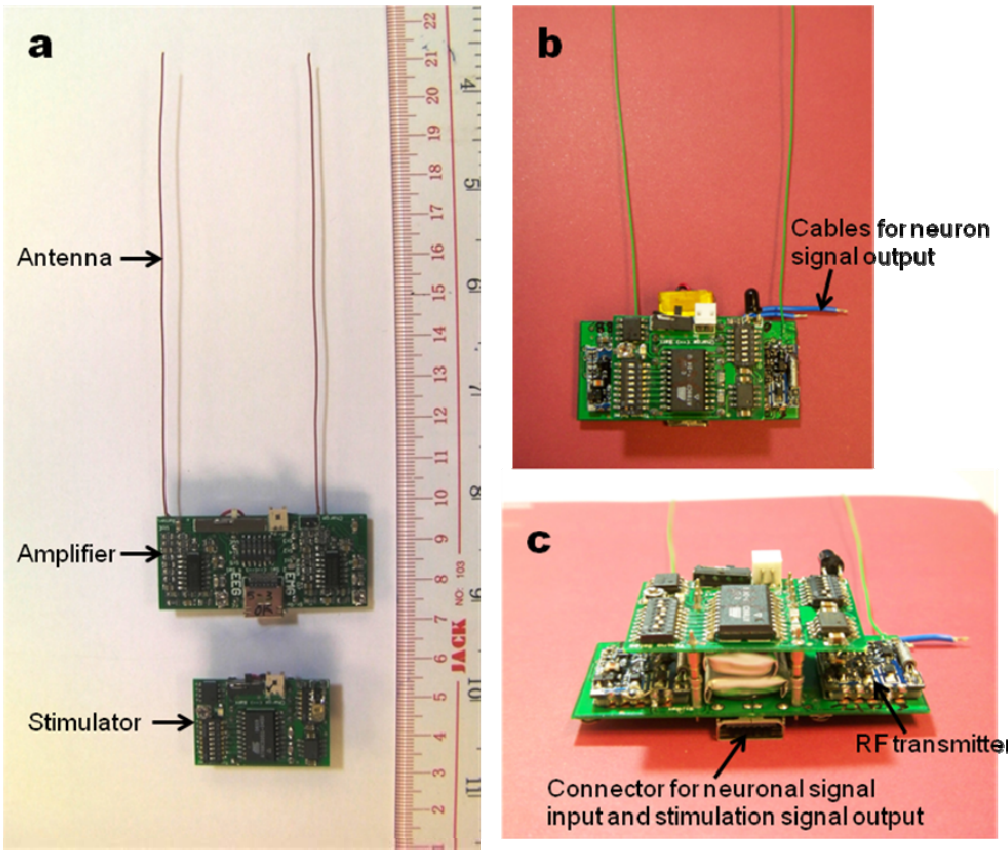

Figure 4.3 The second version of Motolink contains wired and wireless data transmission to computer. a. The amplifier board (50*22 mm) and stimulator board (32*22 mm) are separated and can be connected together by 5 pairs of pins between them. The two antennas were for radio frequency telemetry. **b.** There are outputs of neuronal signal through cables for wired transmission. **c.** A 14-pins connector is the input of neuronal signal and output of stimulation signal which to be held on the head of guinea pig. There are several switches on the circuit for multi-choices selection, hence the circuits is designed to be naked without any cover.

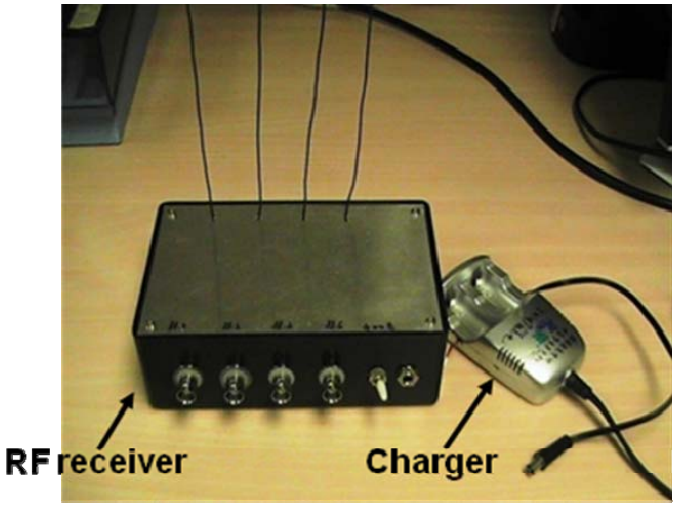

Figure 4.4 RF receiver with four particular frequencies and battery charger for Ver. 2.0 of Motolink. Four particular frequencies are 80.0, 88.2, 88.4, and 89.0 respectively.

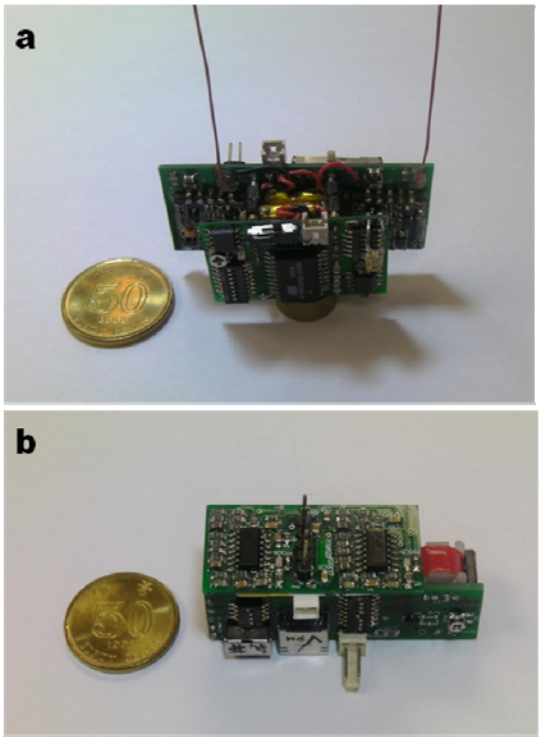

Figure 4.5 Two versions of Motolink are compared with a 50 cents coin of Hong Kong. a. Ver. 1.0 Motolink. b. Ver. 2.0 Motolink.

4.2.2 Multi patterns of intraspinal stimulation

The intraspinal stimulation is generated in the microcomputer unit (MCU) of the stimulator on Motolink. In basic protocol, when there is a neural spike above the threshold, a train of 5 stimulation pulses will be generated 5 ms later. In advanced protocol, there are four patterns of stimulation that will be triggered in given condition. After X signals higher than threshold, with 5ms delay, the stimulation pulses will generate. There are four choices: X= 1, 2, 4, 8. After the first upper-threshold signal, within the time window Y, the number of (X-1) signal should come out (see Figure 4.6 A). There are four choices: Y= 50 ms, 100 ms, 150 ms, 300 ms. The pattern of stimulation pulses is Z, and four choices are Z= a, b, c, d (Figure 4.6 B). The relax time after the last pulse is N, and two choices are N= 50 ms, 100 ms.

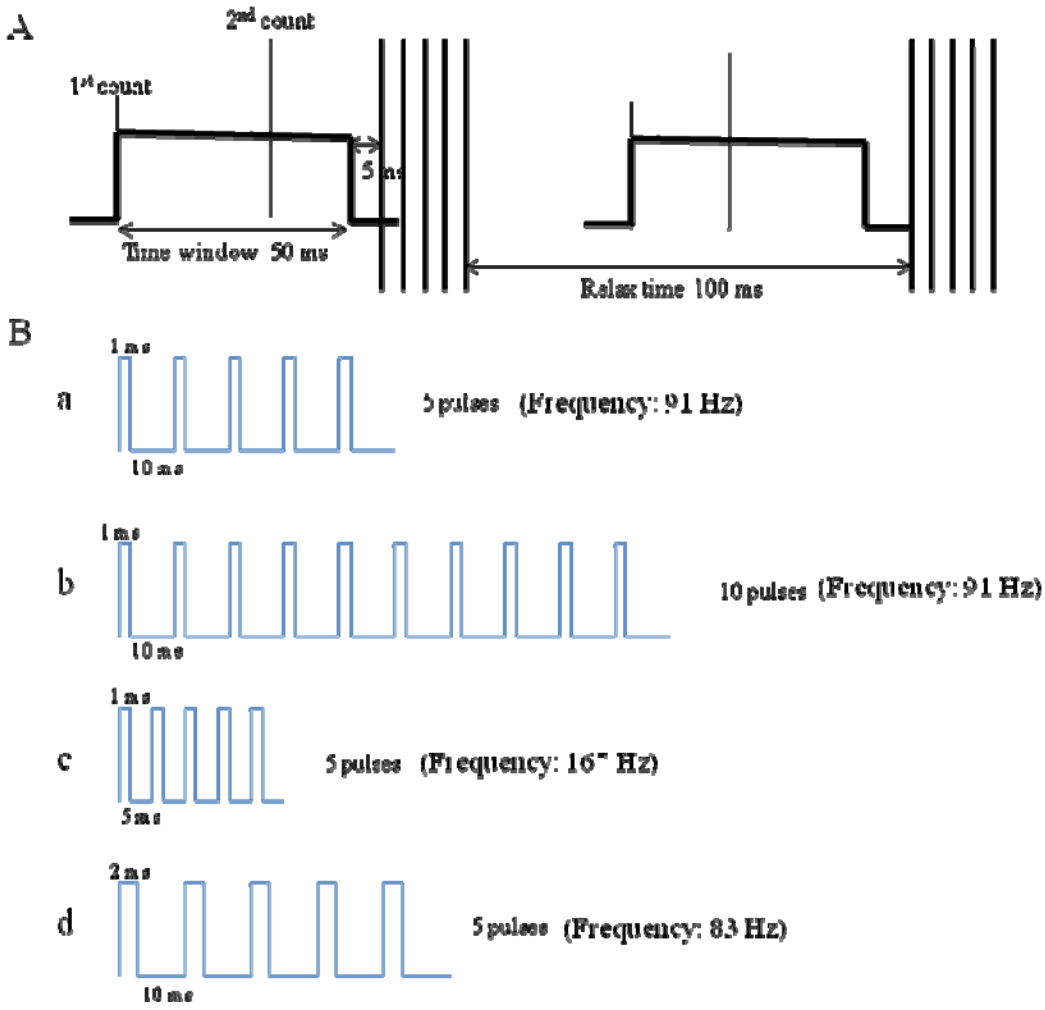

Figure 4.6 Different patterns of intraspinal stimulation. A. Sample of a stimulation pattern: after the first upper-threshold signal, within the time window 50 ms, totally there are 2 signals higher than threshold, with 5ms delay, 5 pulses of stimulation with 91 Hz frequency is generated. After 100 ms of relax time following the last pulse, another train of pulses is generated by next two signals. **B.** Four patterns of stimulation. a. 5 pulses with 91 Hz. b. 10 pulses with 100 Hz. c. 5 pulses with 167 Hz. d. 5 pulses with 83 Hz.

4.2.3 Electrodes array and socket for connection

4.2.3.1 Electrode array of extracellular recording

Three kinds of electrode array have been developed for extracellular recording of the primary motor cortex. One custom-made electrode array was designed in shape of a row (Figure 4.7). It contains four tungsten electrodes (diameter 50 μm, impedance 400 kΩ to 600 kΩ; Institute of Biophysics, Chinese Academy of Sciences, Beijing, China) and spaces 500 μm between electrodes. Tip of electrodes is exposed for 30-50 μm and the length of electrodes sticking out of silicon elastomer is about 2 mm. The ends of electrodes are soldered to the pins of usual connector, which is the output of extracellular signals and convenient for selecting recording position. The electrode array can be embedded into dental acrylic on the skull for fixation of the implantation depth of electrodes. The middle-level charge capacity and very low rate of dissolution in pulsing of this kind of tungsten electrode enable them keep middle-term stability in vivo.

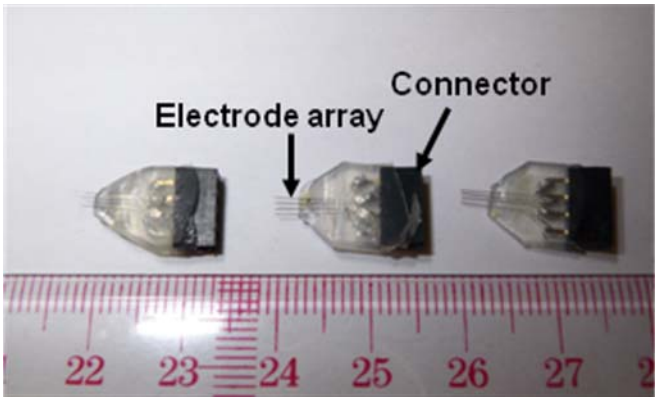

Figure 4.7 Electrode array in a row shape for the extracellular recoding.

Another electrode array with four groups of electrodes was also developed in the lab (Figure 4.8), which containing both recording and stimulating electrodes. Each group has three electrodes, two tungsten electrodes (Diameter 50 μm, impedance 200 kΩ -1 MΩ, A-M system, USA) for extracellular recoding, and a stainless steel electrode (Diameter 50 μm, impedance 30 kΩ -100 kΩ, A-M system, USA) for stimulating. The diagram of this electrode array is demonstrated in Figure 4.8. This electrode array can be used to give intracortical microstimulation to identify the hindlimb area of the primary motor cortex, and synchronously record the response activity of adjacent neurons around the stimulating site. This design improved the accuracy of inserting recording electrodes to the right position.

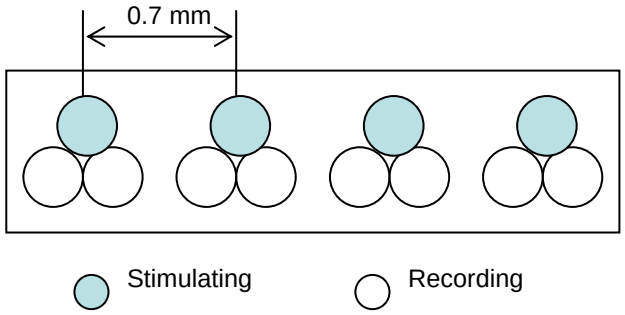

Figure 4.8 Diagram of electrode array with both recording and stimulating electrodes.

In addition, we developed electrode array in matrix (custom-made by the Institute of Biophysics, Chinese Academy of Sciences, Beijing, China) for extracellular recoding (see Figure 4.9). This electrode array only has recording electrodes and can be implanted instantly into the primary motor cortex with minimal lesion by the pneumatically actuated inpulse microelectrode array inserter (Blackrock Microsystems, UT, USA). The electrode array in matrix is manufactured with tungsten microwire

(diameter 50 μm, 200 to 500 kΩ impedance) insulated by Teflon, or platinum mixed with iridium (diameter 30 μm, 500 to 600 kΩ impedance) isolated by parylene. It is designed in 2×3 or 3×3 array, with 0.5 mm interval between electrodes. The length of each electrode sticking out of the silicon elastomer is equivalent, but the length differs from 1.0 to 1.2 mm in different arrays according to different usage. The tips of electrodes are exposed of 10-30 μm.

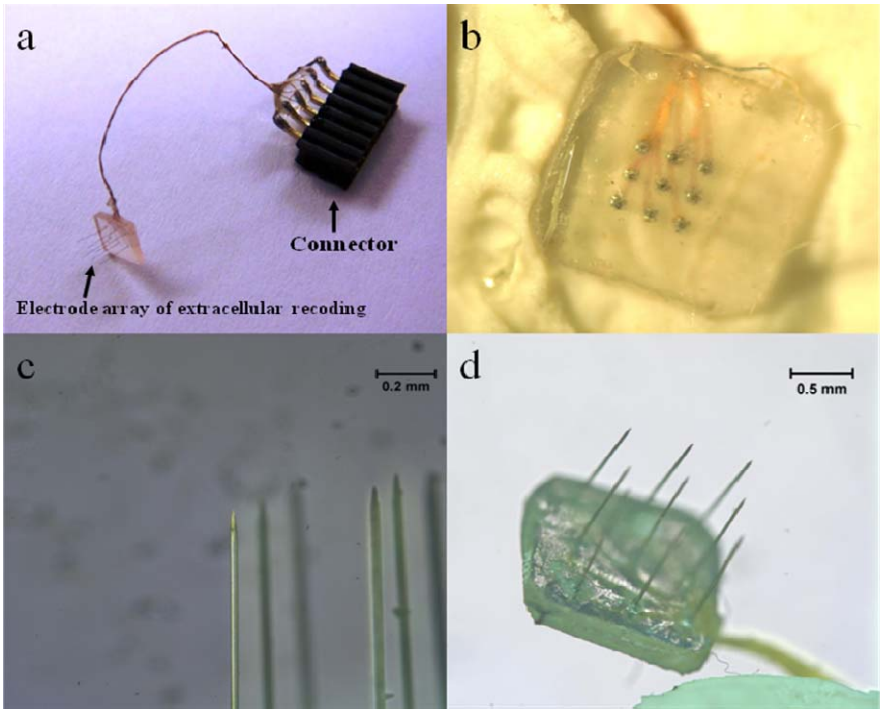

Figure 4.9 Electrode array in matrix for extracellular recoding. a. Electrode array is linked with a usual connector. **b.** Wires connect to electrodes inside the plat. **c.** Close-in of the tip of an electrode, amplification ×100. d. 3×3 electrode array.

4.2.3.2 Electrodes of intraspinal microstimulation and electromyography

Six earphone wires (enamelling copper wire, 75 μm diameter, impedance of 10 to 50 kΩ, cross-section exposed) are soldered to pins of socket as electrodes of

intraspinal microstimulation. One of them is reference electrode and the other five are stimulating electrodes.

Three earphone wires (enamelling copper wire, diameter of 75 μm, and impedance of 10 to 50 kΩ) are used to record the electromyography activity of forelimb. Two wires are recording electrodes and one is reference electrode. A loop of 2 mm diameter was tied at each end of wire for increasing the contact area to muscles.

4.2.3.3 Socket for connection

Socket is the relay station of signal switching between cortical activity, EMG, Motolink, intraspinal stimulation and external computer. A finished product of socket (1 gram) is showed in Figure 4.10. It is connected with output and input by 14 pins and wires. The 14 pins of sockets are connected to the following contents: 5 inputs of extracellular recording in the motor cortex from the electrode array, 3 inputs of electromyography activity from electrodes inserted into forelimb muscles and 6-8 outputs of intraspinal microstimulation through the stimulation electrodes. Additionally, there are two lead-out wires for two outputs for data transmission on the amplifier circuit. On operation, the socket was secured on the skull of subjects by screws and dental acrylic. All microwires were lead subcutaneously from socket to the M1, forelimb and the spinal cord.

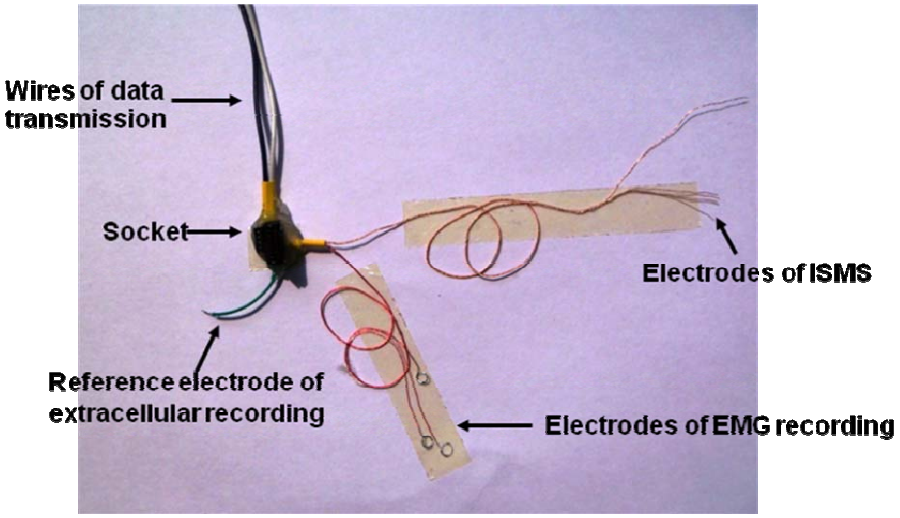

Figure 4.10 Socket with output cables for holding Ver. 1.0 Motolink. It is connected to electrodes of extracellular recording, EMG recording, ISMS and wires for data transmission.

4.2.4 Experimental preparation

4.2.4.1 Subjects

Forty adult Hartley albino guinea pigs (*Cavia porcellus*, weight 400-800g, either sex, SPF) provided by the Chinese University of Hong Kong, were served as subjects in this study (Figure 4.11). Subjects care before experiments and experimental approvals are the same as acute experiments. All experiments were conducted in compliance with the *Principles of Laboratory Animal Care* (National Institutes of Health, No. 86-23, revised 1985) and all experimental procedures were approved by the Animal Subjects Ethics Sub-Committee of the Hong Kong Polytechnic University. The experimenters have gotten the Personal License of Animal Regulations approved by the Department of Health in Hong Kong.

Figure 4.11 Hartley guinea pigs were used as subjects in chronic experiments.

4.2.4.2 Surgical procedures of electrodes array implantation in M1

All surgeries were performed in aseptic conditions. Animals were pretreated with atropine (40-200 pg/ kg, sc; Sigma, USA) to restrain respiratory secretions. Pentobarbital sodium (40 mg/kg, i.p.; Sigma, USA) was administrated and maintained at one-quarter level (10 mg/kg/hr, i.p.) hourly during the operation. For convenience of electrode array implantation, the head, neck, forelimb and lower back of subject were shaved. Subject was mounted on the stereotaxic device and its body temperature was controlled between 37.5 and 38.5 °C by homoeothermic blanket system.

Craniotomy was performed to expose M1 area of the cortex in the right hemisphere of brain, according to the commercial atlas (The rat brain 5^{th}). Microelectrode array in row shape was advanced slowly into the target area of the primary motor cortex by micromanipulator (Narishiga, Japan) to the depths of 1.0-1.5 mm below the surface. Placement of microelectrode array is demonstrated in Figure 4.12. Silicone elastomer (Kwik-Sil, World Precision Instruments, FL, USA) was then covered on the cortex to provide protection. The upper part of electrodes and connector were embedded in dental acrylic so that the depth of electrode was fixed to the cortical

surface relatively.

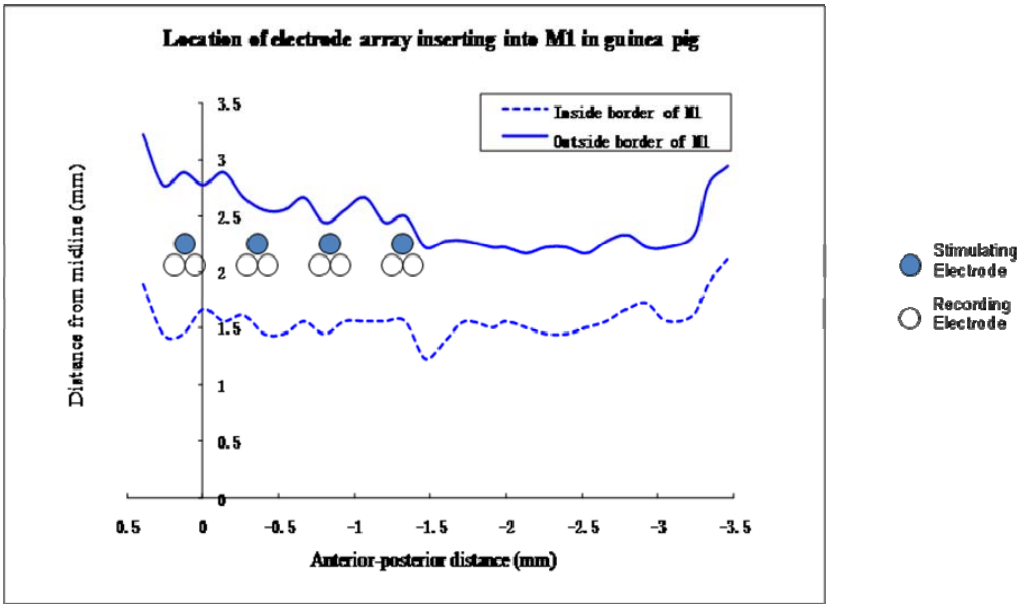

Figure 4.12 Location of the electrode array in the primary motor cortex of guinea pig. Area between the two blue lines indicates part of M1 in rats, which is expanded proportionally according to "The rat brain 5th". Electrode array is implanted into this area.

When electrode array in matrix was used to acquire neural activities from the primary motor cortex, after removing the dura carefully, a stimulation electrode was inserted into the primary motor cortex to detect the area of hind limb with intracortical microstimulation (13 pulses, 333 Hz, 60~200 μA, cathodal pulses, isolated constant current). Positive locations were verified by hindlimb response to microstimulation (Kleim, Barbay et al. 1998; Karl, Sacrey et al. 2008). Simple verification of hindlimb area of the primary motor cortex was conducted each time before electrodes implantation. After confirmation, electrode array in matrix was driven into the cortex instantly by pneumatically-actuated inpulse inserter (Blackrock Microsystems, UT, USA), which facilitates complete array implantation with minimal tissue damage

(Figure 4.13). Then the electrode array was embedded in silicone elastomer for protection and fixation. The wire bundle which connects electrodes and connector, and the lower part of connector were all embedded in dental acrylic in the end. Whole procedure of surgery is demonstrated in Figure 4.14.

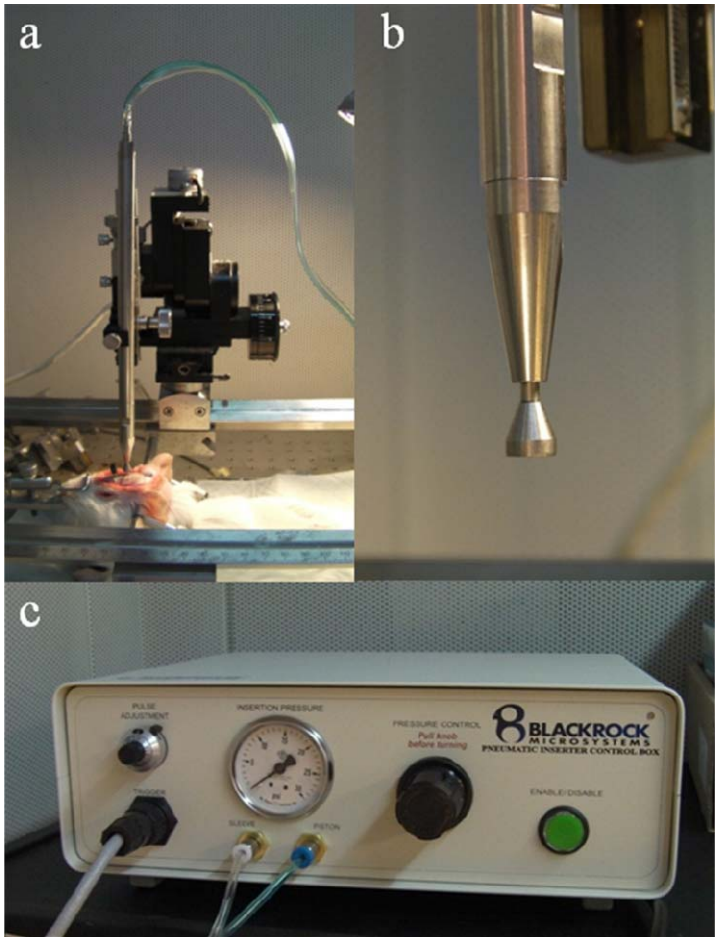

Figure 4.13 Pneumatically-actuated inpulse inserter is used for electrode array implantation. a. The head of inverter vertically above the electrode array is ready for pneumatical actuation. **b.** Close-in of the head. **c.** The pneumatical control box.

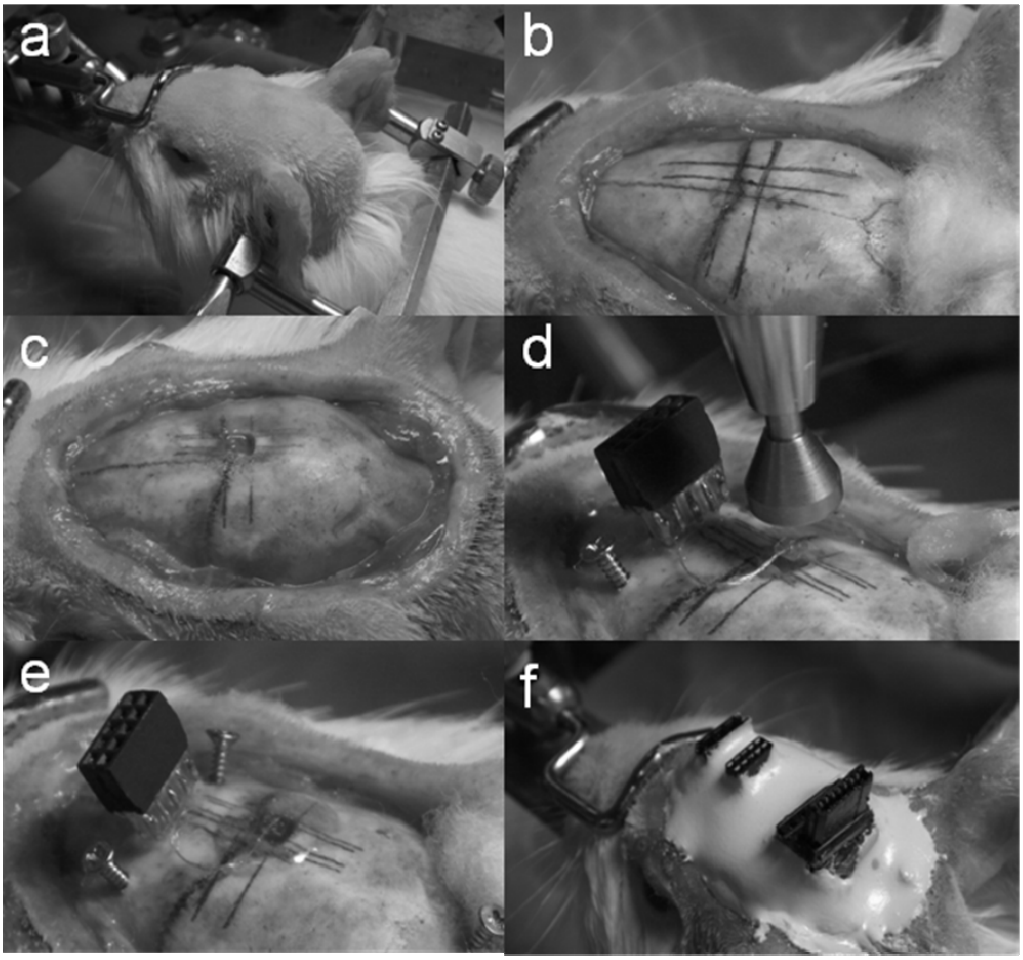

Figure 4.14 Procedure of implantation of cortical electrodes array and socket embedment. a-c. Craniotomy. **d-e.** The electrode array is implanted pneumatically by inserter. **f.** The connectors of electrode array and socket are embedded in dental cement.

4.2.4.3 Surgical procedures of electrodes array implantation in the spinal cord

The techniques of electrode implantation and stabilization were based on those developed for chronic recordings from neurons in spinal dorsal roots in cats for the neurophysiologic studying of movement (Lemon 1984; Mushahwar, Collins et al. 2000). The skin of lower back, left hindlimb and left forelimb were shaved beforehand. A socket attached with intraspinal stimulation electrodes and forelimb EMG recording electrodes was secured to the skull by dental acrylic. The spinal cord of guinea pigs was transected completely by micro scissor at the level of T12. The dorsal surface of vertebral L1 to L2 was removed to expose spinal cord segments L2 to L3. Six microwires were led subcutaneously from head to L2-L3 spinal cord. A reference electrode was placed in the erector spinae muscles nearby. After removing dura carefully, five wires were vertically inserted into left L3 segment respectively with electrical stimulation to target the regions concerning locomotion of left lower limb in guinea pigs, which was located in the ventral horn of spinal cord. The position of target was referred to acute experiments conducted previously. In case the regions were confirmed, the depth of microwires was kept stable in silicone elastomer and the flat parts of wires were anchored to the L1 spinous process by dental cements. The whole procedure of implantation of microwires in the spinal cord is showed in Figure 4.15.

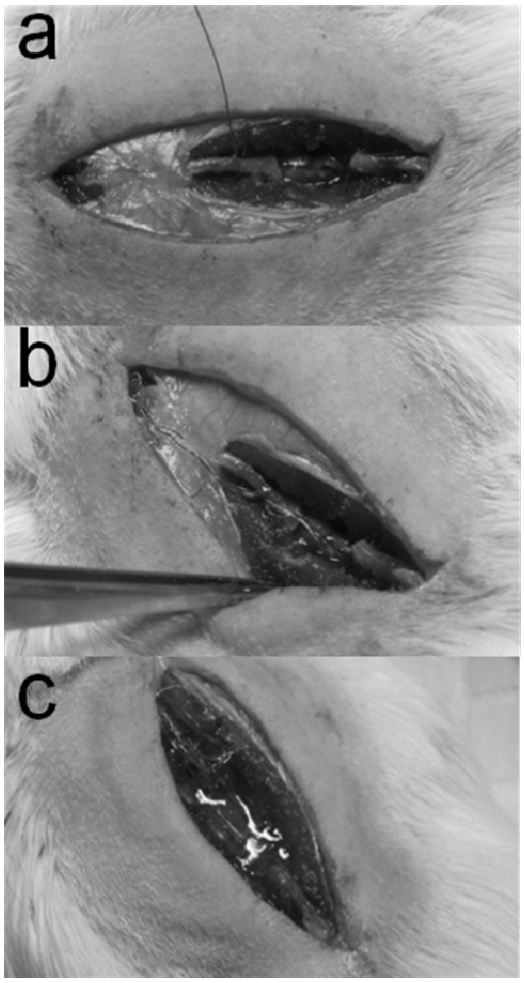

Figure 4.15 Procedure of electrode array implantation in the spinal cord. a. The spinal cord is exposed by rachiotomy. **b.** The microwires bundle is attached to previous spinous process and the microwires are inserted carefully into the spinal cord. **c.** The electrode array of stimulation is embedded and fixed by silicone elastomer.

4.2.4.4 Surgical procedures on electrodes implantation of electromyogram

To record EMG activity of fore limb muscles, pairs of microwires were intramuscularly inserted in the triceps brachii. Triceps brachii is the muscle of lateral surface of the front leg, which involves in elbow extension and forelimb adduction during the movement. A reference electrode was inserted into the tendon of triceps brachii near the elbow joint. Microwires were lead subcutaneously to socket on skull.

At the end of operation the spinal opening and fore limb incision were sutured carefully.

4.2.4.5 Postoperative nursing of animals

After surgery animals were allowed to rest on electrical homothermous pad on the first day to keep body warmth. Analgesia of buprenorphine was induced in a dose of 0.1 mg/kg i.s. and maintained for 3 days, 2 times a day. Wound was administrated with unguentun erythromycin (Baiyunshan Inc. Guangzhou, China) for 3 days daily, 3 times a day. Penicillin (50000 units/kg i.m., 1 time per day) was administrated as antibiotic if there was an infection. Animals were allowed 5-7 days for recovery before terminal experiments. The postoperative nursing of animal was performed intensively in the first 3 days and two times per day in the following days, containing abdomen and hindlimb massage, assistance of micturition and wound cleaning. Subject usually can survive for about 1 month. If there will be severe infection or serious discomfort in animals during survival period, they were received overdose sodium pentobarbital (60 mg/kg, i.p.) for euthanasia.

4.2.4.6 Training of animal walking on the treadmill

After 5-7 days recovery from surgery operation, subjects were trained to walk on the treadmill by forelimbs at different speeds. The speeds increased gradually from 5.6 cm/s to 11.1 cm/s. In warm-up section, animal practiced to walk on the treadmill at low speed for 5 minutes. Then in recording section, the performance of animal was

recorded in trails. Each trail lasted for 1 minute and animal was allowed to rest for another 1 minute. After 3 trails of stimulation-off recording and 3 trails of stimulation-on recording (totally 6 trails), the speed of treadmill was increased to next level. The maximum speed of treadmill was determined by the condition of subjects on the experimental day.

4.2.5 Date collection

4.2.5.1 Cortical extracellular recording

Experiments on awake animal were carried out from 7 days after surgery. Animal was trained to walk by their forelimb on treadmill at a comfortable and constant speed (5.6 cm/s -16.7 cm/s) when it was suspended by lower limb harness. The extracellular signals and artifacts caused by electrical stimulation in the spinal cord were stored to computer disk together for later analysis. Collecting data from subjects with Motolink on treadmill walking often lasted at most 1 hour, which depended on the conditions and tolerance of subjects. Extracellular signals were amplified 3000-4000 times by Motolink, recorded by Axon Digidata 1440A and Axoscope 10.0 (Molecular Devices Co., Chicago, IL) with band-pass filtered by 300-5000 Hz and sampling rate of 10000 Hz.

4.2.5.2 Electromyography recording

EEG activity of forelimb movement was recorded by the other amplifier simultaneously when it walked on the treadmill. EMG signals were amplified 1000-

2000 times by Motolink, recorded by AxoDigidata 1440A and Axoscope 10.0 (Molecular Devices Co., Chicago, IL) with band-pass 30-1000 Hz and sampling rate of 10000 Hz.

4.2.5.3 Kinematic recording

Real-time movement of the limbs was monitored by video (Resolution 720×576, GZ-MG27AH, JVC, Japan). A 3-D motion capturing system (Vicon workstation, Version 370/Version 3.2 Build 049, eight cameras system, LA, CA) was used to monitor kinematics of animals walking on the treadmill at the same time. Three Vicon video cameras operated at 60 Hz, 60 frames per second. Retro-reflective markers (diameter 10 mm) were attached to the wrist of left forelimb and the ankle of left hindlimb. These markers were used to define the relative position between limbs during movement. There were only videos recorded from free moving guinea pigs, without Vicon recording.

4.2.5.4 Histology analysis

At the end of the study, subject was deeply anesthetized with an overdose of pentobarbital sodium (0.1 ml/100 g, 60 mg/ml, i.p.; Sigma). Small electrical lesions (9V constant current for 2-4 min or 1 mA constant current for 1-3 min) marked the location of electrodes for histological identification through recording electrodes in M1 and stimulating electrodes in the spinal cord. After electrical lesion, subjects were perfused transcardially with 400 ml 0.9% NaCl, followed by 400 ml ice-cold 4%

paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brain and the spinal cord were quickly removed from skull and spinal column. After post-fixation by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4), and dehydration by 30% sucrose in 0.1 M phosphate buffer (PB, pH 7.4), frozen section was conducted. The coronal sections (40 μm) of the brain containing recording sites were sliced. As well as the coronal sections (40 μm) of the spinal cord containing the stimulation sites and the sagittal sections (30 μm) of the spinal cord containing the lesion site were sliced respectively. After complete dryness, Nissl staining was performed to these sections. Photography (SPOT Hex, Diagnostic instruments, inc., USA) by microscope (Eclipse 80i, Nikon, Japan) of the sections with Nissl staining was taking and stored for histology analysis of the tip of recording electrodes in the primary motor cortex and the lesion of transection.

4.2.6 Mapping of hindlimb area of the primary motor cortex

Right side of the primary motor cortex in guinea pig was mapped by electrical microstimulation. This experiment was conducted acutely. Nice adult Hartley albino guinea pigs (weight 400-800g, either sex, SPF), *Cavia porcellus*, provided by the Chinese University of Hong Kong, were served as subjects in this study. Subjects care before experiments and experimental approval are identical to the ones for acute experiments in chapter three.

Subject was mounted on the stereotaxic device. Animals were pretreated with atropine (40-200 pg/ kg, sc; Sigma, USA). Pentobarbital anesthesia (40 mg/kg, i.p.; Sigma, USA) was administrated during surgical preparation and maintained at one-

quarter level (10 mg/kg/hr, i.p.) per hour during whole operation. Animal's body temperature was controlled between 37.5 and 38.5 °C by homoeothermic monitoring system during the course of surgery. Craniotomy was performed to expose the cortex in the right hemisphere of the brain. After removing dura carefully, microelectrode array was advanced slowly by a micromanipulator (100 microns/min) to the depths of 1.5 mm below the surface in the hindlimb area of the primary motor cortex.

A train of 13 pulses stimulation (square wave with isolated constant current, 0-250 μA, frequency of 333-350 Hz) was generated by TDT system and Isolator (). The stimulation was delivered to the cortex through tungsten electrode of low impedance (10-100 kΩ, FHC Inc., Bowdoin, ME) to evoke hindlimb movements. Each site of stimulation was distributed with 0.3 mm intervals from rostral to caudal and 0.3 mm intervals from midline to lateral cortex. Reference electrode was placed in the cortex nearby the Bregma. In case contralateral hindlimb has response to the stimulation, which could be observed by eyes, this site was considered as positive site and the location of stimulating was recorded immediately. Otherwise, it was not in the hindlimb area of primary motor cortex. Videos of the response of locomotion were recorded simultaneously by camera (Resolution 720×576， GZ-MG27AH, JVC, Japan).

At the end of experiments, subjects were sacrificed by overdose of pentobarbital sodium (60 mg/kg, i.p.).

4.2.7 Data analysis and statistics

Data were analyzed using Axon Clampfit 10.0 (Molecular Devices Co., Chicago, IL) and SPSS. The Student's t-Test was used for statistics analysis of time lag between LFL and LHL, and data are given as Mean +- SD.

4.3 Results

4.3.1 Mapping of hindlimb area of the primary motor cortex

By intracortical microstimulation in acute experiments, the representation of different body parts in the primary motor cortex has been investigated. Only forelimb areas, trunk areas and hindlimb areas were mapped here. A general map integrated from mapping result of four guinea pigs is shown in Figure 4.16. We found that the hindlimb area of primary motor cortex is located mainly in the posterior part of the precentral cortex. The region of M1HL locates approximately 1.5 to 3 mm lateral from midline, 0.5 to 3.5 mm posterior from Bregma. And the forelimb area of primary motor cortex (M1FL) is primarily located in the anterior part of the primary motor cortex. The area of M1FL ranges from 1.5 to 4 mm lateral from midline, and 0 to 2 mm anterior from Bregma.

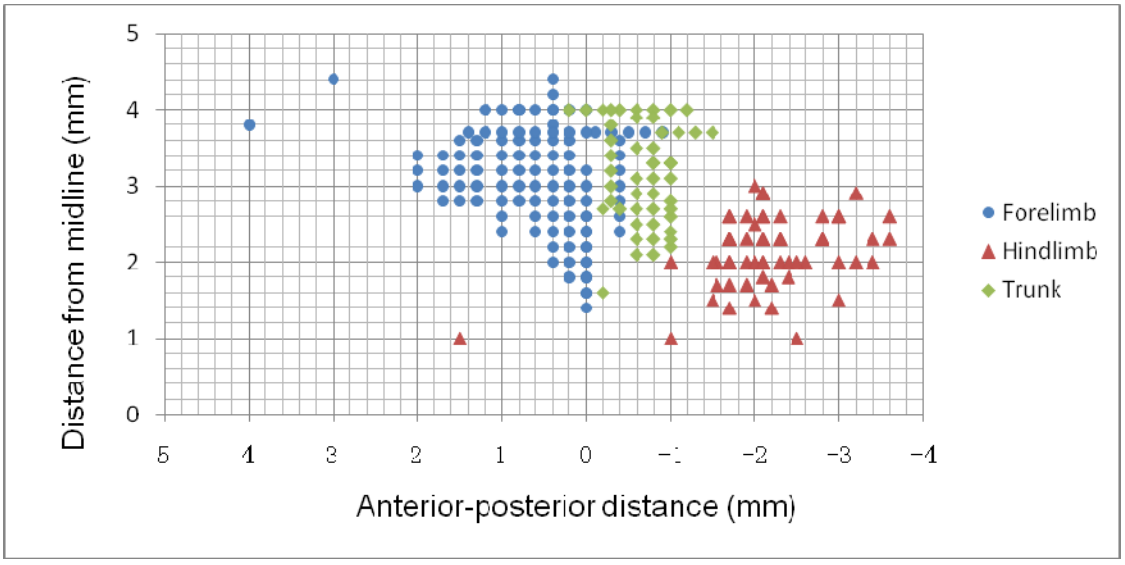

Figure 4.16 Composite representation of primary motor cortex from four guinea pigs. The stimulation is given as a train of 13 pulses with current 60-250 μA, 333 Hz.

Most EMG activities of four muscles in which we inserted the electrodes were generated by stimulation with upper-threshold current (Figure 4.18). Regular EMG activity was recorded as given 60 μA current, which was the threshold current (Figure 4.18 c). The amplitude and the duration of EMG activity corresponded to the current intensity (Figure 4.19). The electrically activated response of hindlimb or forelimb was recorded by videos respectively when giving intracortical microstimulation to corresponding M1. Synchronized video of the forelimb response see Movie 4-1 (Figure 4.17_Movie, 9 s); synchronized video of the hindlimb response see Movie 4-2 (Figure 4.17_Movie, 6 s). Movies are attached in the supplementary disk. Movie 4-1 shows forelimb response by ICMS (100 μA, 13 pulses, 333 Hz); movie 4-2 reports hindlimb response by ICMS (200 μA, 13 pulses, 333 Hz).

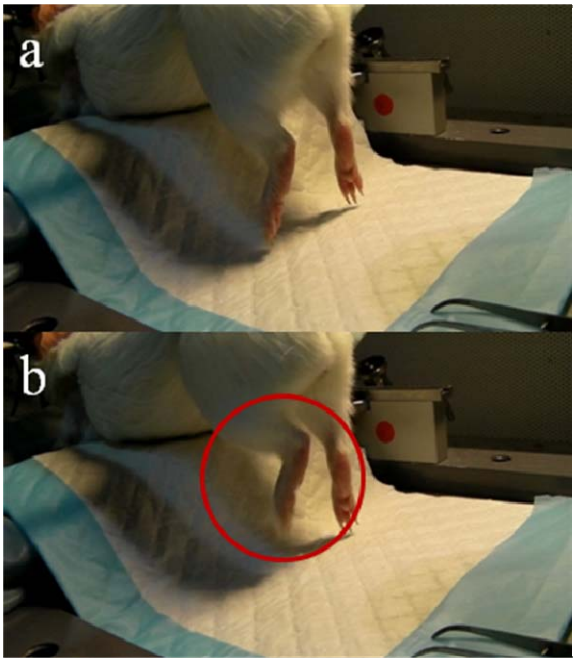

Figure 4.17 The response of contralateral hindlimb as giving microstimulation in the related primary motor cortex. a. The hindlimb is relaxed. **b.** Hindlimb responded to the intracortical microstimulation, red circle indicates the contraction. Synchronized video of the forelimb is in Movie 4-1 and synchronized video of hindlimb response is in Movie 4-2: Figure 4.17_Movie.

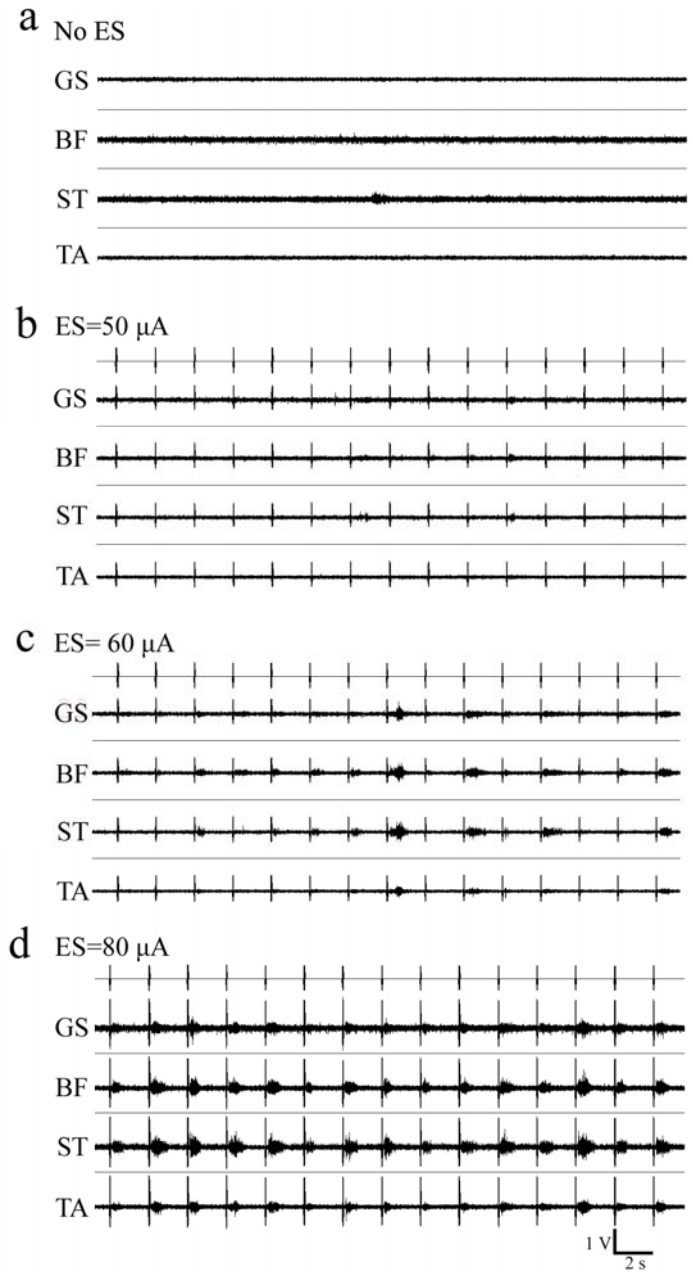

Figure 4.18 EMG activity of left hindlimb was activated by upper-threshold current. a. There was no EMG activity at all when no current. **b.** Irregular EMG responded to stimulus was recorded from four electrodes inserted in different muscles as given 50 μA current. **c.** Regular EMG activity was recorded as given 60 μA current, which was the threshold current. **d.** EMG activity was obviously recorded as the current increased to given 80 μA. ES: electrical stimulation, GS: gluteus superficialis, BF: biceps femoris, ST: semitendinosus, TA: tibialis anterior.

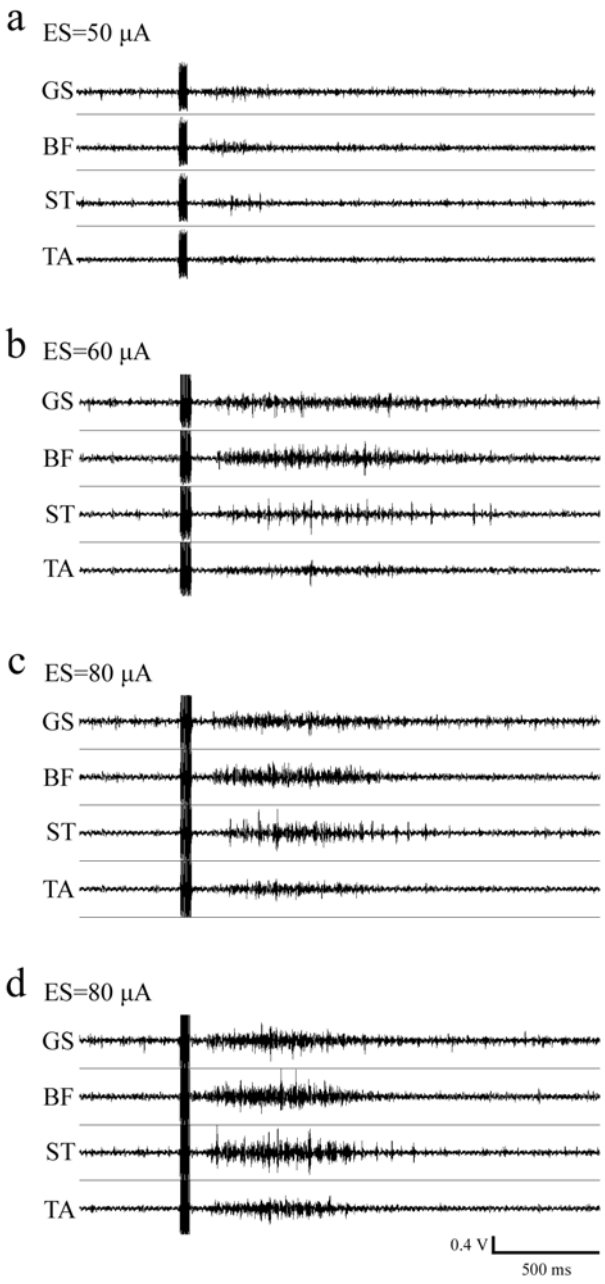

Figure 4.19 The character of EMG activity varied as the current intensity increased. a. the amplitude of EMG activity was small as given 50 μA current, **b.** the amplitude of EMG activity increased but the duration was long (more than 100 ms) as given 60 μA current, **c-d.** the amplitude of EMG activity increased significantly but the duration was shorten to 60 ms around as given 80 μA current. ES: electrical stimulation, GS: gluteus superficialis, BF: biceps femoris, ST: semitendinosus, TA: tibialis anterior.

4.3.2 Reconnection of upper motor neurons and lower motor neurons when guinea pig walked on the treadmill with wires for signal transmission

4.3.2.1 Histological analysis of the recording site in the primary motor cortex, the lesion in the spinal cord and the location of stimulation electrode

After application of cortical neuronal signals from AC and VC, as well as neuronal signals from MGB, we want to apply neuronal activity of upper motor neurons in the primary motor cortex to be reconnected with lower motor neurons in guinea pigs with spinal cord lesion. Animal with Motolink was walking on the treadmill when we recorded data Figure 4.20 a). Extracellular electrodes were implanted in layer V of the right M1 (marked with Nissl staining, Figure 4.20 b) responsible for motor control of the left hindlimb (LHL). The stimulation electrodes and electromyography electrodes that were implanted in the left forelimb were tunneled under the skin to the Motolink. Nissl staining showed the stimulating electrodes were implanted in the motor neurons pool of spinal cord (Figure 4.20 e). The lesion is complete by transection and there is no nerve connection in the spinal cord (Figure 4.20 c).

An electrode array of 4-6 electrodes was implanted in the motor cortex. Motor signals could typically be acquired from at least 2-3 electrodes. No further analysis was performed to classify the signals. A bunch of 6-8 stimulation electrodes were implanted into the spinal cord. Typically, 3-4 of these electrodes were effective. Data were obtained with only one recording channel and one stimulation channel. Behavioral testing was performed on forty guinea pigs. Thirty six animals were examined on the treadmill test, while four were examined in free-moving experiments.

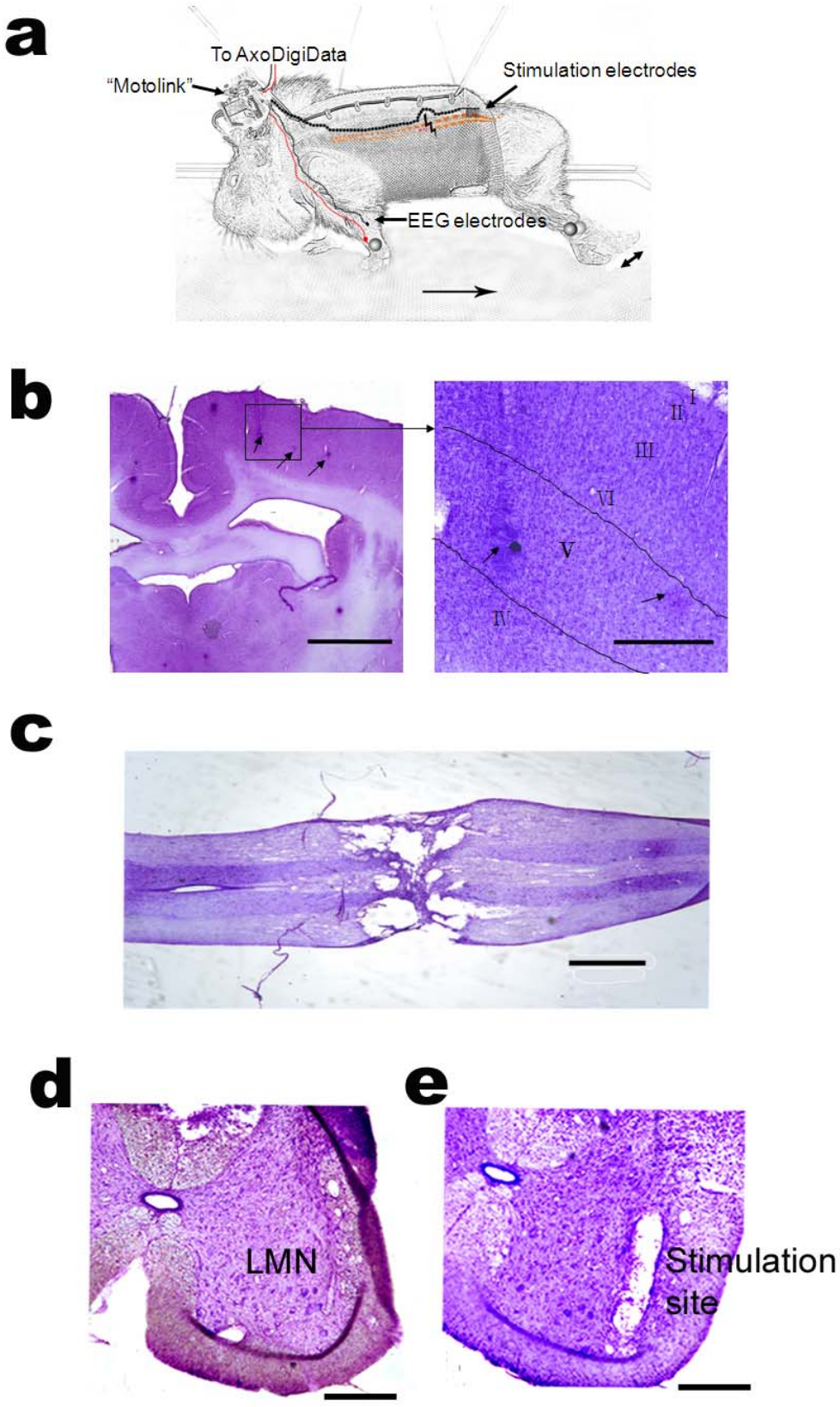
a
To AxoDigiData
"Motolink"
Stimulation electrodes
EEG electrodes
b
I
II
III
VI
V
IV
c
d
LMN
e
Stimulation
site

Figure 4.20 Upper motor neurons reconnected with lower motor neurons in awake, behaving guinea pigs. a. A schematic drawing of the experimental setup. A recording electrode array was implanted into the motor cortex, and a pair of EMG electrodes was implanted in the left forelimb, and six to eight stimulation electrodes were placed in the spinal cord targeting the hindlimb region (L3-4). The amplifier and the programmed stimulator were fixed on the skull with a socket. The triggered movement of left hindlimb is indicated by a double arrow. **b.** Nissl staining shows the recording sites in layer V of M1 which is indicated by arrows. Left panel: The electrode traces was observed in the cortex. Scale bar, 1 mm. Right panel: From the cortical layer structure, we could identify the primary motor cortex, with its giant upper motor neurons in the layer V. Scale bar, 200 μm. **c.** The lesion by complete transection 21 days after surgery of animal #1 is demonstrated by Nissl staining of cord slice. Scale bar, 1 mm. **d.** Motor neurons locates in the ventral horn of L3 spinal cord. Scale bar, 200 μm. **e.** A stimulation electrode is located in the motor pool of L3 spinal cord via electrical lesion through the stimulating electrode. Scale bar, 200 μm.

4.3.2.2 Subjects walking on the treadmill at speed of 5.6 cm/s

At the speed of 5.6 cm/s, the extracellular signal of the hindlimb region in M1 showed a rhythmic activity, synchronized with the EMG of the left forelimb (LFL) (Figure 4.21 a). The trajectory of LHL showed irregularly low-amplitude movement in the vertical direction due to running as the stimulator was turned off (Figure 4.21 b). Once the stimulator was turned on, the extracellular cortical signal triggered a 5-pulse train when the signal reached the setting threshold (0.2 V), as indicated in Figure 4.21 c-e. During a sample of 17.0 s walking on the treadmill, 5 of 28 steps (marked with double-arrows) were missed, while one odd-step (marked with the single-arrow) was found in the left hindlimb (Figure 4.21e-f). These missing and odd steps can be observed from the artifacts of electrical stimulation on extracellular and EMG signals clearly. Movement of the left hindlimb was preceded by movement of the left forelimb in time lag of 153+/-42 ms (Mean+/-SE, n=10). (Figure 4.21 f). The left hindlimb showed rhythmic stimulated response which had a good temporal coordination with the left forelimb (Video 4-3: Figure 4.21 e_ Movie, 17 s).

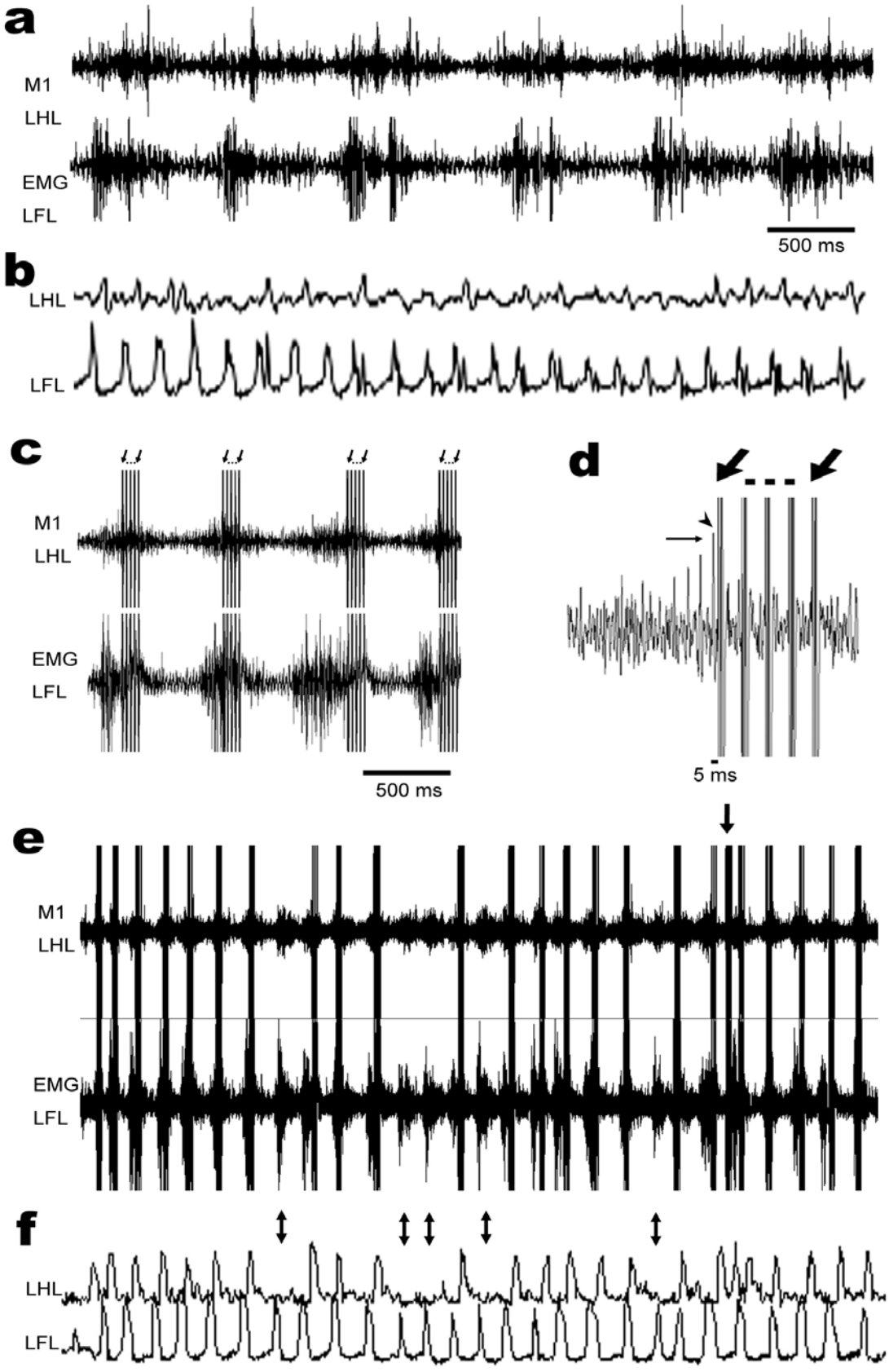
a
M1
LHL
EMG
LFL
500 ms
b
LHL
LFL
c
M1
LHL
EMG
LFL
500 ms
d
5 ms
e
M1
LHL
EMG
LFL
f
LHL
LFL

Figure 4.21 Primary motor cortex active rhythmically and triggered stimulation regularly when animal (subject #1) walked on the treadmill at speed of 5.6 cm/s. **a.** Extracellular neuronal signal (upper panel) from the left hindlimb (LHL) region of the right motor cortex (M1) and EMG signal (lower panel) from the muscle group for extension in the left forelimb (LFL) were recorded while the animal was walking on the treadmill at a speed of 5.6 cm/s. Signals were recorded when the stimulator was switched off as control data. **b.** Kinematic trajectory of LHL and LFL in vertical direction detected by Vicon as control data. **c.** Signals from the same electrodes (M1 and EMG) recorded from the same subject when the stimulator was switched on. Electrical stimulation of the LHL generated artifacts in the recording electrodes, as marked with oblique arrows. **d.** The trigger threshold of the neuronal signal was set at 0.2 V and is marked with a horizontal arrow. The time interval between the trigger signal and the electrical stimulation was set at 5 ms. **e.** Recording of neuronal and EMG signals was sampled when the subject was walking on the treadmill at 5.6 cm/s in 17 s. **f.** Kinematic trajectory of LHL and LFL in vertical direction detected by Vicon is corresponding to the upper panel. A movie of the corresponding episode is attached in Video 4-3: Figure 4.21 e_Movie.

Neural activity of hindlimb area in M1 was filtered (300 Hz to 3 kHz) and rectified for onset analysis. EMG of forelimb was filtered (30 Hz to 500 Hz) and rectified as well (**Figure 4.22**). The onset of neural signal and EMG were determined by the methods proposed in previous research (Yakovenko, McCrea et al. 2005)). Time leg between neural activity of hindlimb area in M1 and EMG of forelimb was 89+/-60 ms.

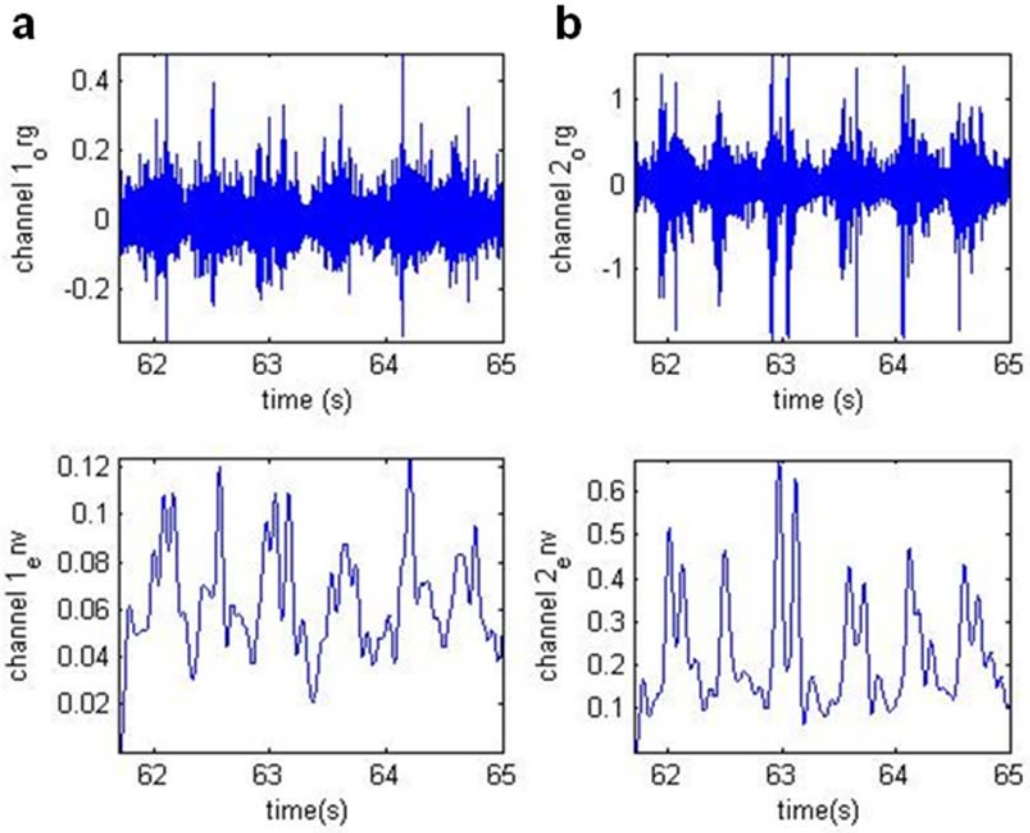

Figure 4.22 Filtered and rectified signals of neural activity and EMG when animal (subject #1) walked on the treadmill at speed of 5.6 cm/s. a, neural activity; b, EMG of forelimb.

Two additional examples of treadmill tests at the speed of 5.6 cm/s after successful implantations are shown in Figure 4.23 and Figure 4.24. A corresponding movie of the hindlimb movement of subject #3 is attached as Video 4-4: Figure 4.24 c_Movie, 20 s. No video of the movement of subject #2 is attached.

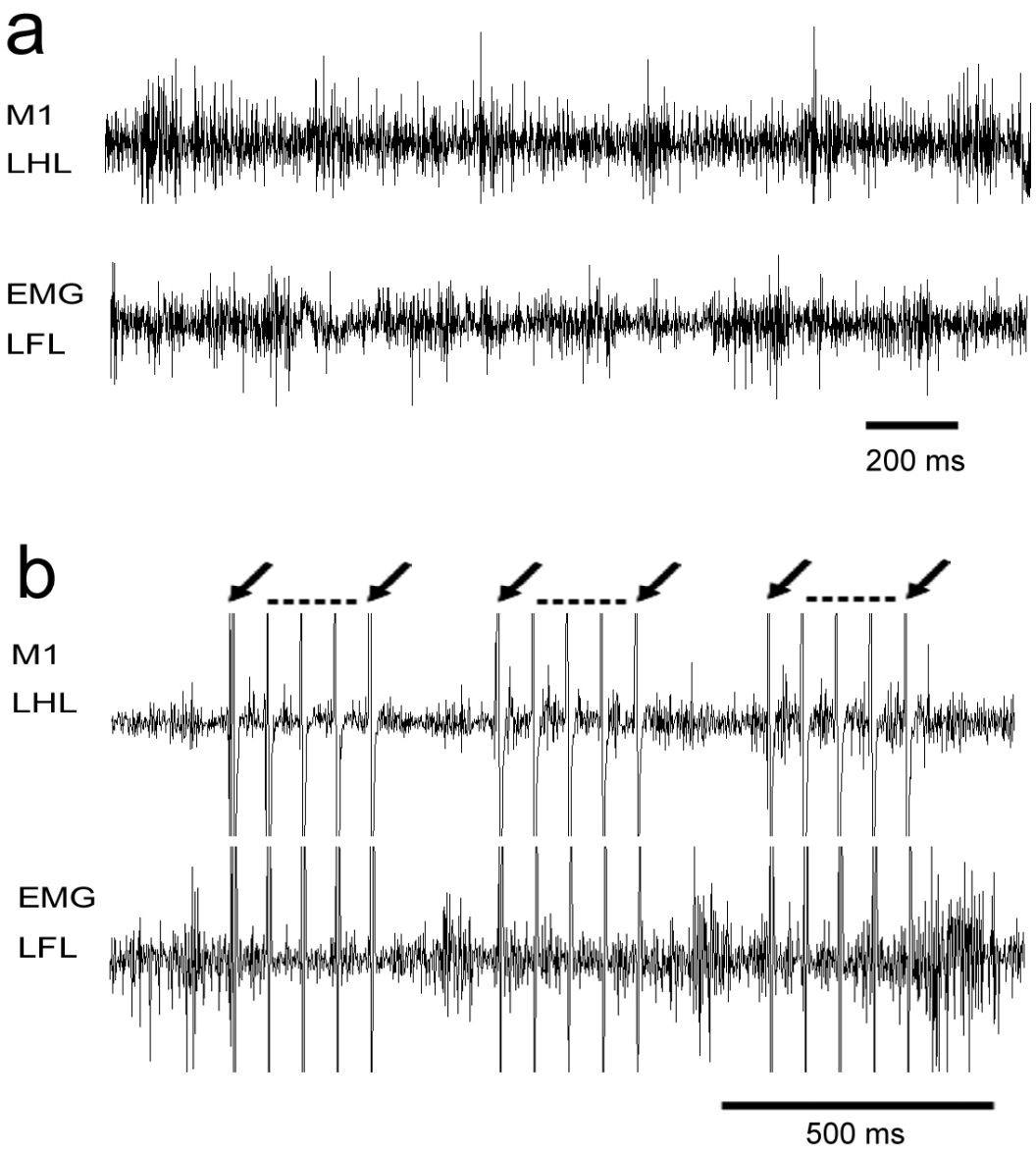

Figure 4.23 Upper motor neurons reconnected with lower motor neurons in awake, behaving guinea pig (subject #2) when walking on the treadmill at speed of 5.6 cm/s. a. Extracellular neuronal signal (upper panel) from the LHL region of the right M1 and EMG signal (lower panel) from the muscle group for extension in the LFL were recorded when the stimulator was switched off. **b**. Signals from the same electrodes (M1 and EMG) recorded from the same subject when the stimulator was switched on. Electrical stimulation of the LHL generated artifacts in the recording electrodes, as marked with oblique arrows.

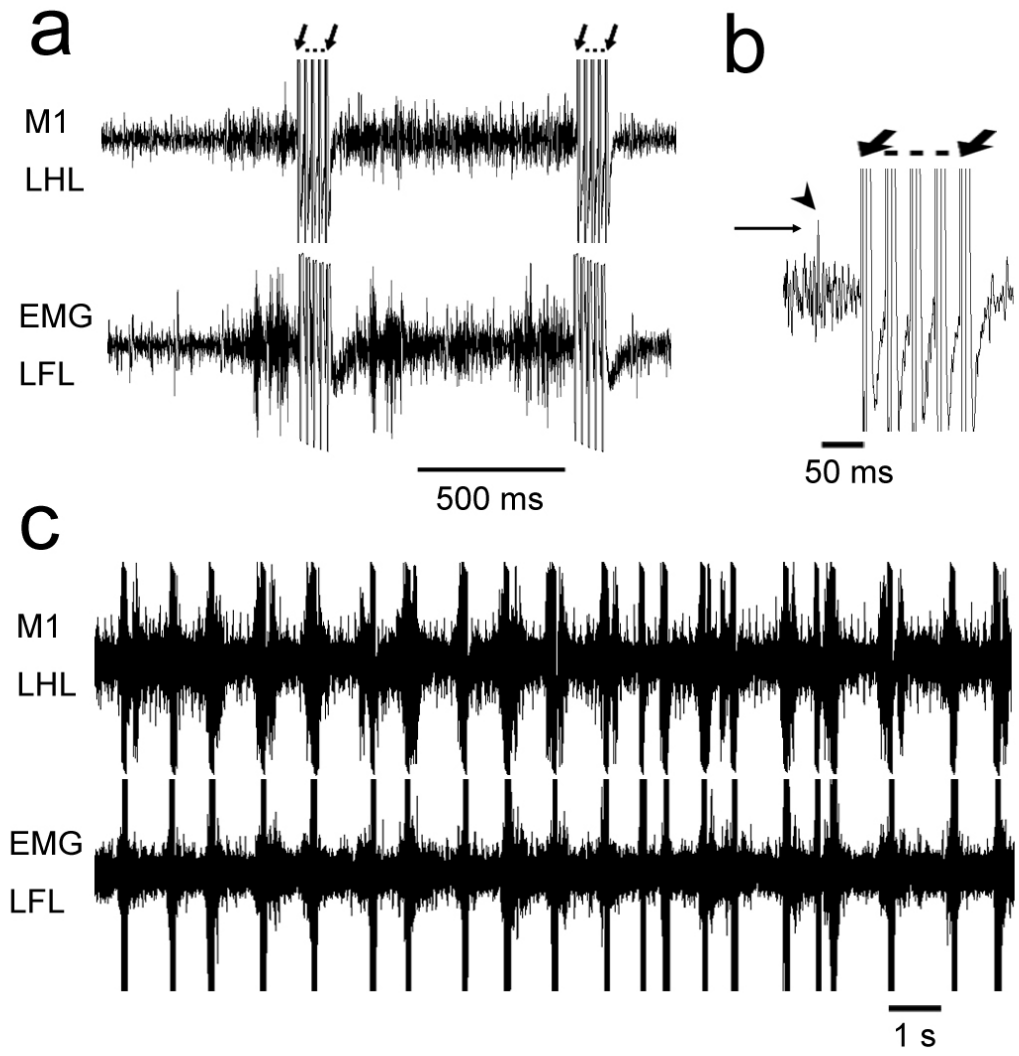

Figure 4.24 Upper motor neurons reconnected with lower motor neurons in awake, behaving guinea pig (subject #3) when walked on the treadmill at speed of 5.6 cm/s. a. Extracellular neuronal signal (upper panel) from the LHL region of the right M1 and EMG signal (lower panel) from the muscle group for extension in the LFL were recorded when the stimulator was switched on. **b.** The trigger signal is marked with an arrow head. The time interval between the trigger signal and the electrical stimulation was set at 50 ms. **c**. A 21 s-episode of recordings of neuronal and EMG signals was sampled when the subject was walking on the treadmill at 5.6 cm/s. Movements of the LHL and LFL in the vertical direction are shown corresponding to the signals. A movie of the corresponding episode is attached as
Video 4-4: Figure 4.24 c_Movie.

4.3.2.3 Subjects walking on the treadmill at speed of 11.1 cm/s

At a faster speed at 11.1 cm/s, the stepping rhythm became faster (Figure 4.25 e-f compared with Figure 4.21 e-f). The extracellular signal of the hindlimb region in M1 showed a rhythmic activity, synchronized with the EMG of the left forelimb (Figure 4.25 a). The trajectory of left hindlimb showed irregularly low-amplitude movement in the vertical direction due to running as the stimulator was turned off (Figure 4.25 b).Once the stimulator switch was turned on, the extracellular cortical signal triggered a 5-pulse train when the signal reached the triggering threshold (0.2 V), as indicated in Figure 4.25 c-d. Five of 39 steps were not detected during the 15.0 s of walking, as indicated by double-arrows in Figure 4.25 e-f. Two odd electrical stimulations were spotted in the middle of the walking period, as indicated by a single arrow (Figure 4.25 e-f). These missing and odd steps can be observed from the artifacts of electrical stimulation on extracellular and EMG signals clearly. Movement of the left hindlimb was preceded by movement of the left forelimb (Figure 4.25 f). The time lag between the forelimb and hindlimb was 92+/-23 ms. The left hindlimb showed a good temporal coordination with the left forelimb (Video 4-5: Figure 4.25 e_ Movie, 16 s). The results are representative of movement achieved in guinea pigs when they walked on the treadmill at speed of 11.1 cm/s.

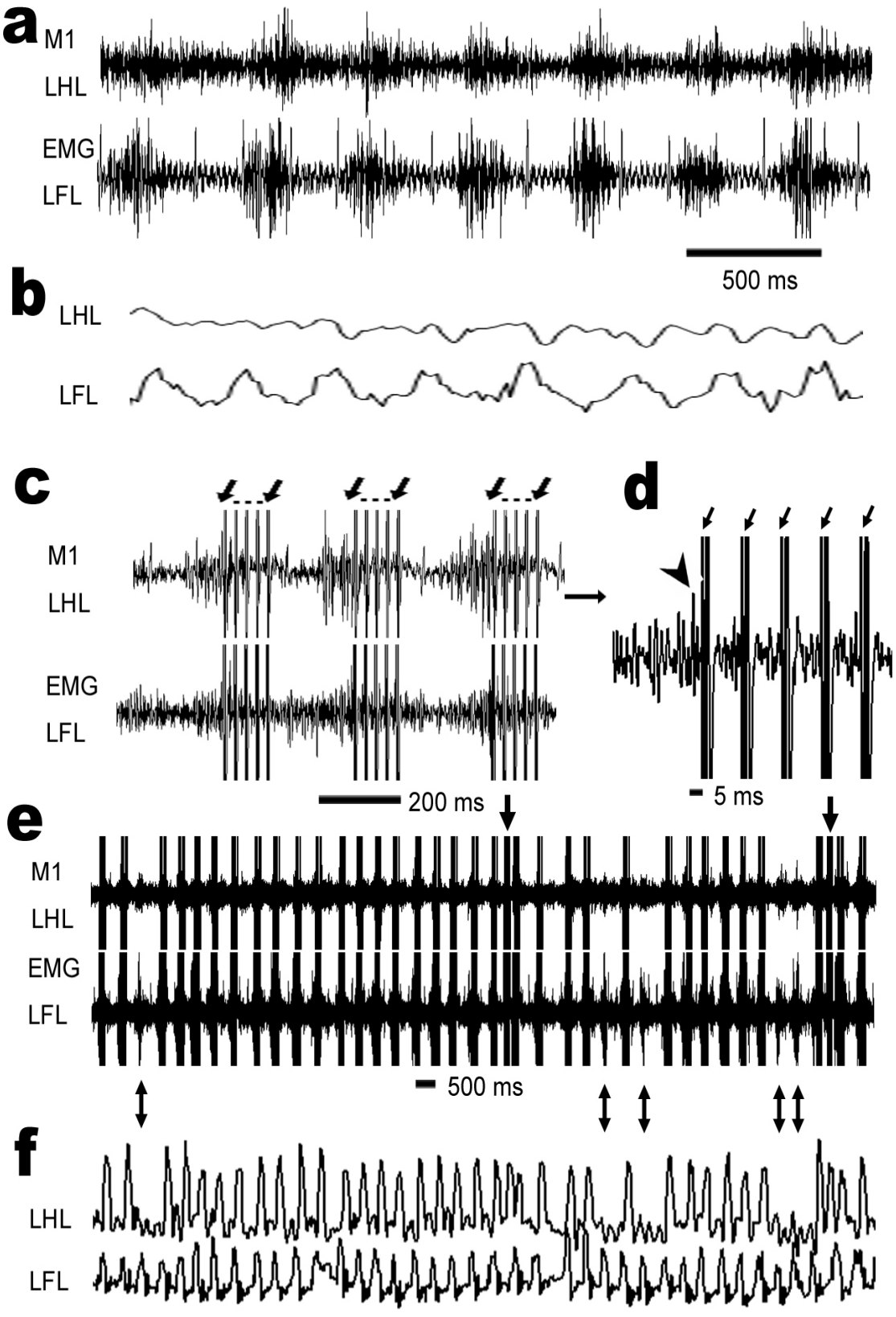
a
M1
LHL
EMG
LFL
500 ms
b
LHL
LFL
c
M1
LHL
EMG
LFL
200 ms
d
5 ms
e
M1
LHL
EMG
LFL
500 ms
f
LHL
LFL

Figure 4.25 An SCI guinea pig (subject #1) walking on the treadmill at different speeds connected to the Motolink. a. Extracellular neuronal signal (upper panel) from the left hindlimb (LHL) region of the right motor cortex (M1) and EMG signal (lower panel) from the muscle group for extension in the left forelimb (LFL) were recorded while the animal was walking on the treadmill at a speed of 11.1 cm/s. Signals were recorded when the stimulator was switched off as control data. **b.** Kinematic trajectory of LHL and LFL in vertical direction when the stimulator was off as control data. **c**. When the stimulator was switched on, electrical stimulation of the LHL generated artifacts in the recording electrodes, as marked with arrows. **d**. The trigger threshold of the neuronal signal was set at 0.2 V and is marked with an arrow. The time interval between the trigger signal and the electrical stimulation was set at 5 ms. **e.** A 15 s-episode of recordings of the neuronal and EMG signals was sampled when the subject was walking on the treadmill at 11.1 cm/s. **f.** Kinematic trajectory of LHL and LFL in vertical direction detected by Vicon is corresponding to the upper panel. A movie of the corresponding episode is attached in Video 4-5: Figure 4.25 e_Movie).

Neural activity and EMG signals were filtered and rectified as well for onset analysis (**Figure 4.26**). Time leg between onset of neural activity of hindlimb area in M1 and EMG of forelimb was 51+/-53 ms.

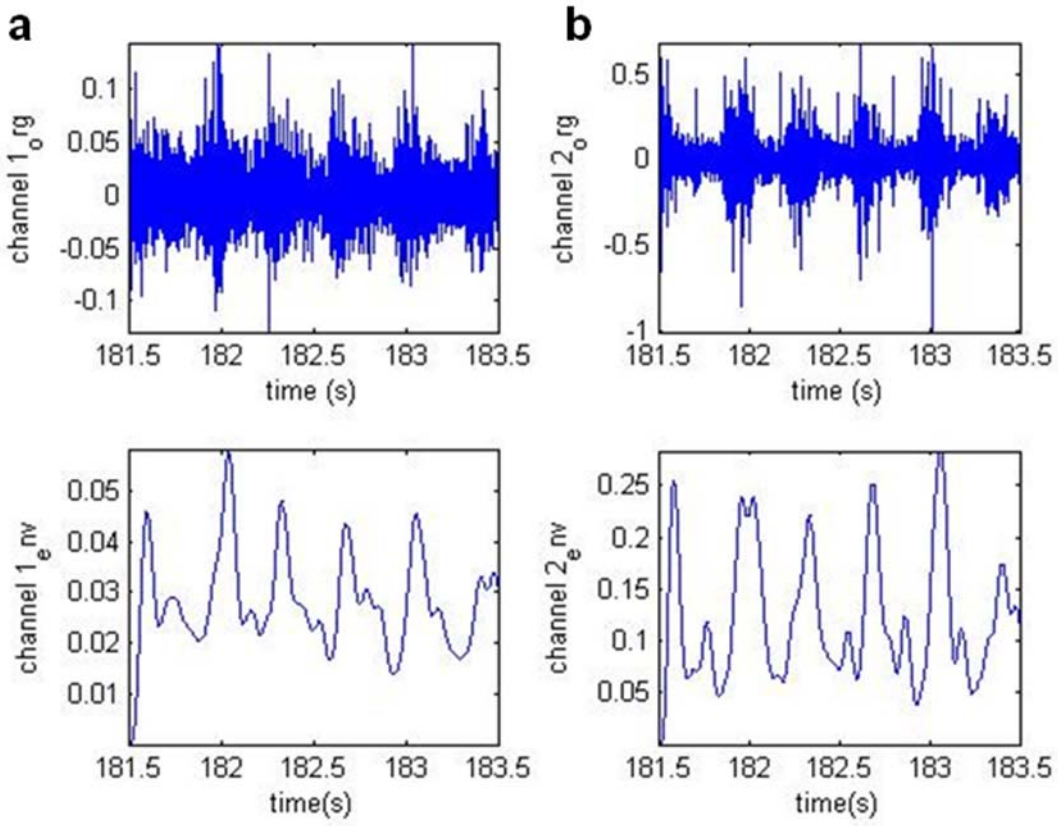

Figure 4.26 Filtered and rectified signals of neural activity and EMG when animal (subject #1) walked on the treadmill at speed of 11.1 cm/s.

In pre-stim steps, the time lag between cortical activity of the hindlimb and EMG signal of the forelimb was shortened significantly from 89+/-60 ms to 51+/-53 ms ($p<0.01$, t-test) when the speed of the treadmill was increased from 5.6 cm/s to 11.1 cm/s (**Figure 4.27**). With stimulation, the time interval between cortical activity of the hindlimb and EMG signal of the forelimb significantly decreased from 77+/-41 ms to 45+/-9 ms ($p<0.01$, t-test).

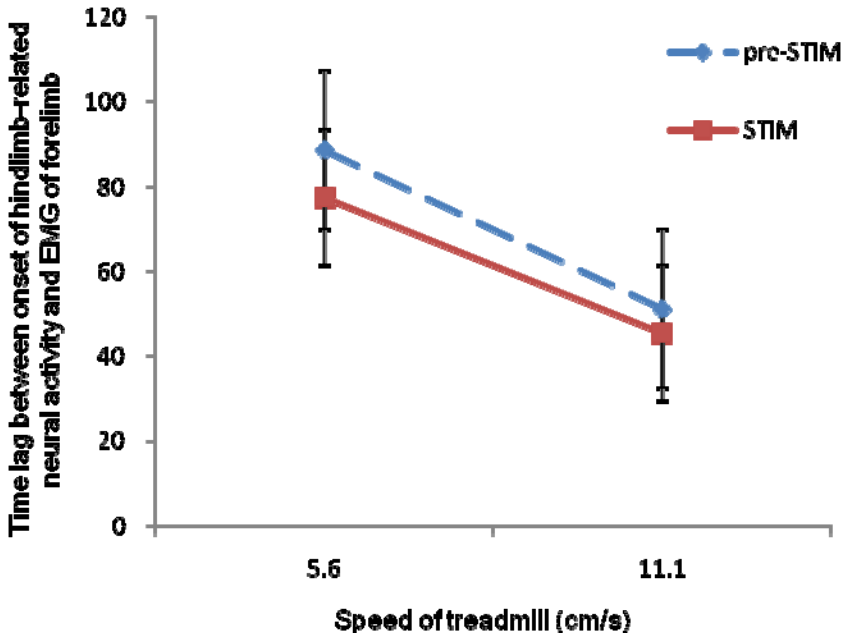

Figure 4.27 Time lag between hindlimb-related neural activity and EMG signal of forelimb decreased as speed of treadmill increased. N=36

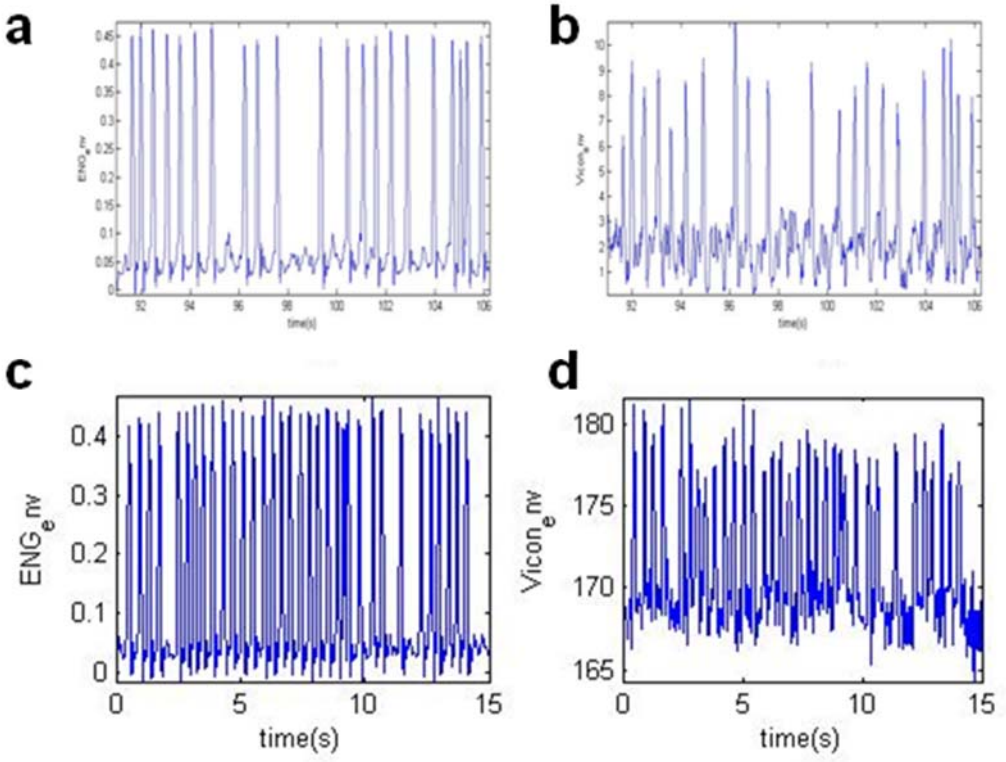

Figure 4.28 Filtered and rectified signals of neural activity and hindlimb trajectory when animal (subject #1) walked on the treadmill at speed of 5.6 and 11.1 cm/s. a and b, 5.6 cm/s; c and d 11.1 cm/s; a and c, neural activity of hindlimb area in M1; b and d, hindlimb trajectory.

Neural activity and Vicon signals were filtered and rectified as well for onset analysis (**Figure 4.28**). When we compared the time lag between onset of cortical activity of the hindlimb and elicited movement of the forelimb by stimulation (**Figure 4.29**), we found there was not significant shortened from 84+/-21 ms to 70+/-21 ms ($p<0.01$, t-test) when the speed of the treadmill was increased from 5.6 cm/s to 11.1 cm/s.

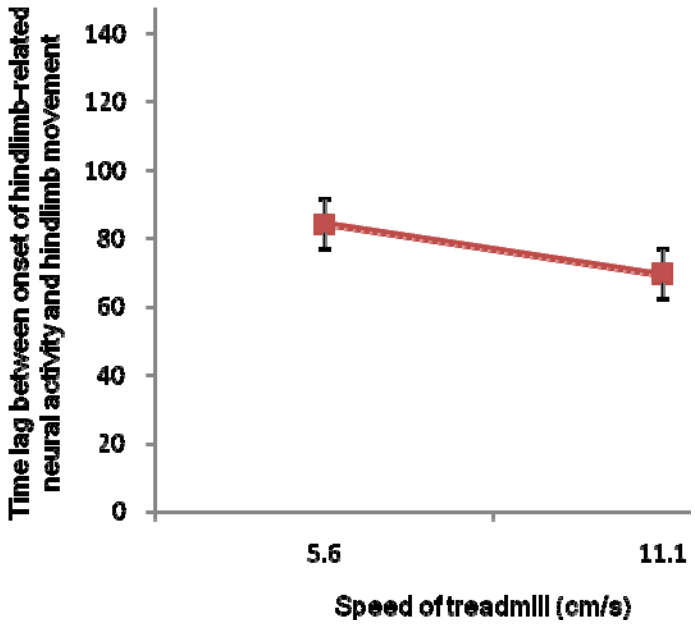

Figure 4.29 Time lag between hindlimb-related neural activity and hindlimb movement decreased as speed of treadmill increased. N=35

The time lag between the forelimb and hindlimb was shortened significantly from 153+/-42 ms to 92+/-23 ms ($p<0.01$) when the speed of the treadmill was increased from 5.6 cm/s to 11.1 cm/s. This shortened time lag indicated an increased stepping speed. The details of the other two subjects connected with Motolink and tested on the treadmill are shown in Figure 4.23 and Figure 4.24. The time lag between the left forelimb and hindlimb of the three subjects tested on the treadmill is shown in Figure 4.30. In all subjects, there was a shortened time lag between the intact left forelimb and the stimulated hindlimb when the speed of the treadmill was increased from 5.6 cm/s to 11.1 cm/s, and in one subject when the speed was increased from 11.1 cm/s to 16.7 cm/s.

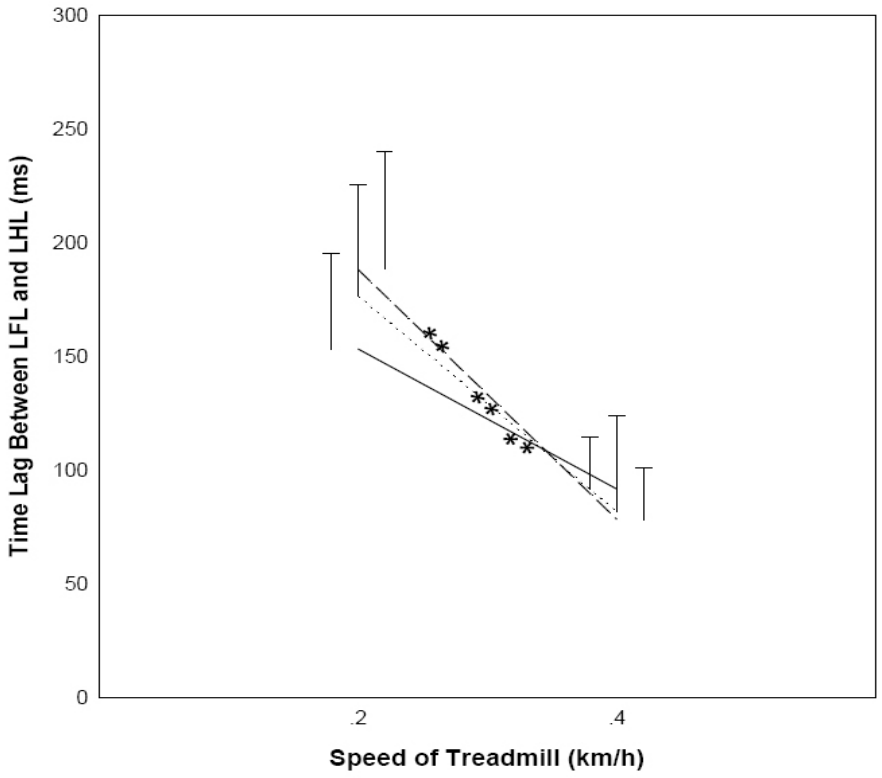

Figure 4.30 Time lag of movements of the intact left forelimb and the stimulated left hindlimb of paraplegic guinea pigs walking at different speeds on the treadmill. Results were sampled from three subjects at three different speeds on the treadmill. Note: 0.2 km/h equals to 5.6 cm/s; 0.4 km/h equals to 11.1 cm/s. Results were averaged over ten steps for each sample. Statistics were compared between different speeds for each individual subject (** $p<0.01$, t-test).

When we compared extension amplitude of left hindlimb during movement, there is significant difference between the pre-stim steps and stim situation (** $p<0.01$, t-test). Difference between left forelimb before and after stimulation is not as significant as hindlimb group (* $p<0.05$, t-test).

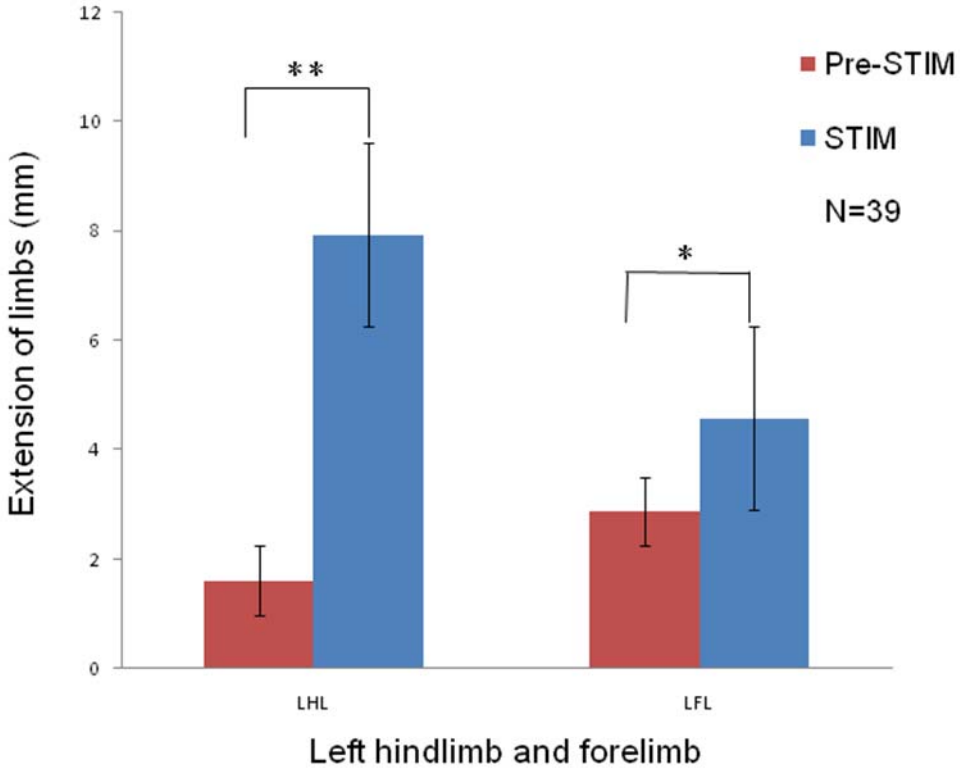

Figure 4. 31 Comparation of the extension of left hindlimb and forelimb.before and after stimulation.

4.3.2.4 Subjects behaving freely

To test the reconnection of UMNs and LMNs in freely behaving condition, four additional subjects were examined. The animal showed no movement of the left hindlimb when it was not moving, but started to twitch when it started to move (Figure 4.32). A representative video is attached as Video 4-6 (Figure 4.32 c_Movie, 12 s). Twitching was a typical movement caused by 5-pulse SC electrical stimulation (see Movie 4-7 filmed in close-up, 15 s).

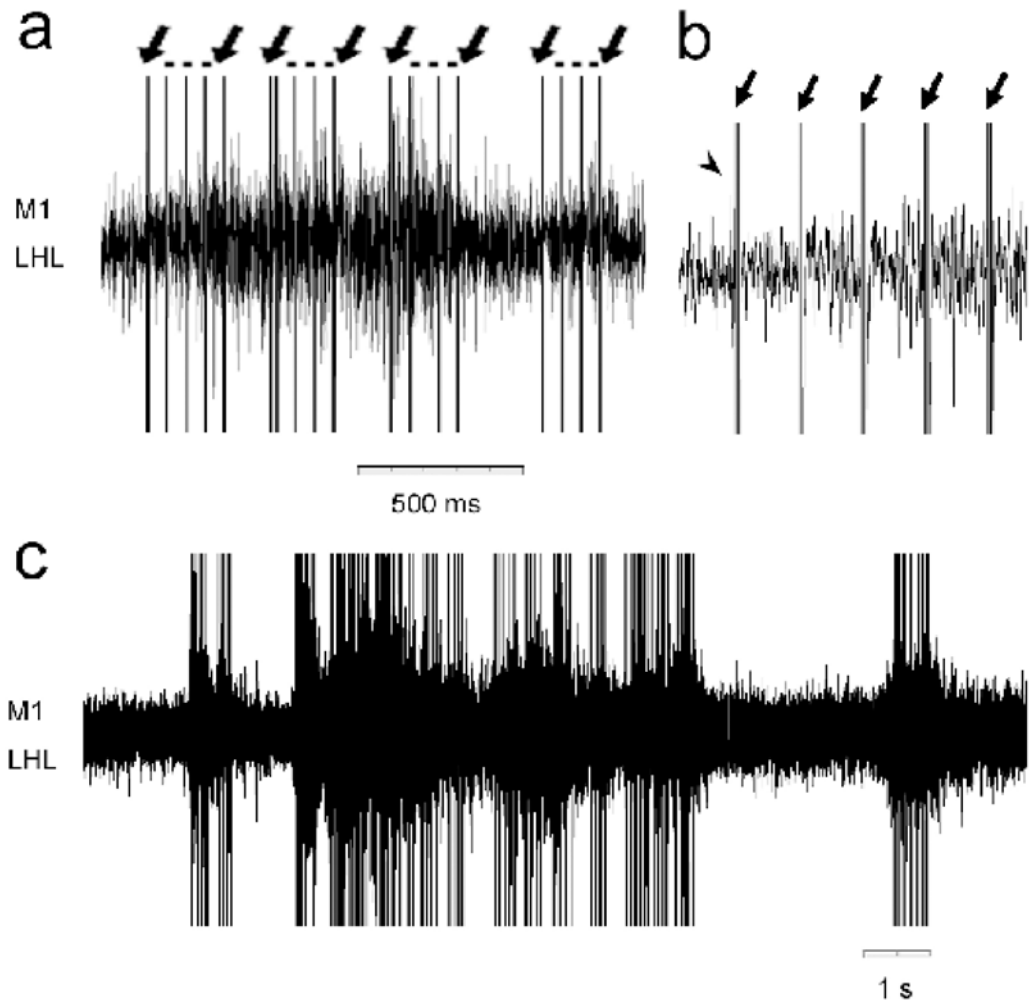

Figure 4.32 Upper motor neurons were reconnected with lower motor neurons in freely behaving guinea pig (subject #4). a. Extracellular neuronal signal (upper panel) from the left hindlimb (LHL) region of the right motor cortex (M1) was recorded while the animal was behaving freely, when the stimulator was switched on. **b.** Electrical stimulation of the LHL generated artifacts in the recording electrodes, as marked with arrows. The trigger threshold of the neuronal signal was set at 0.2 V. **c.** A 15 s-episode of recordings of the neuronal signals was sampled when the subject behaved freely. A movie of the corresponding episode is attached in Video 4-6: Figure 4.32 c_Movie.

4.3.3 Reconnection of upper motor neurons and lower motor neurons when animal walking on the treadmill with signal transmission wirelessly

4.3.3.1 Stimulation patterns generated by stimulation protocol

To generate correspondent stimulation based on different neuronal commands, multi protocols of stimulation were designed. As mentioned in Figure 4.6, four patterns of stimulation were triggered by spikes of M1. Four stimulation pulses were 40 Hz, 5 pulses (Figure 4.33 a); 91 Hz, 5 pulses (Figure 4.33 b, c, f); 167 Hz, 5 pulses (Figure 4.33 d); 91 Hz, 10 pulses (Figure 4.33 e). Either one (Figure 4.33 a), two (Figure 4.33 b-e) or four spikes (Figure 4.33 f) could trigger stimulation. The time window of signal selection is 50 ms (Figure 4.33 b, e, f) or 100 ms (Figure 4.33 c-d). Selection of different stimulation protocol depends on the number of spikes occurred in the step rhythm and the frequency of discharge. For example, if there are 2 spikes higher than threshold appear in the hindlimb region of M1 on each step during forelimb movement, and these 2 spikes mostly occur in 50 ms on each step, we choose stimulation pattern 1, the 5-pulse stimulation with frequency of 91 Hz produces (Figure 4.33 b).

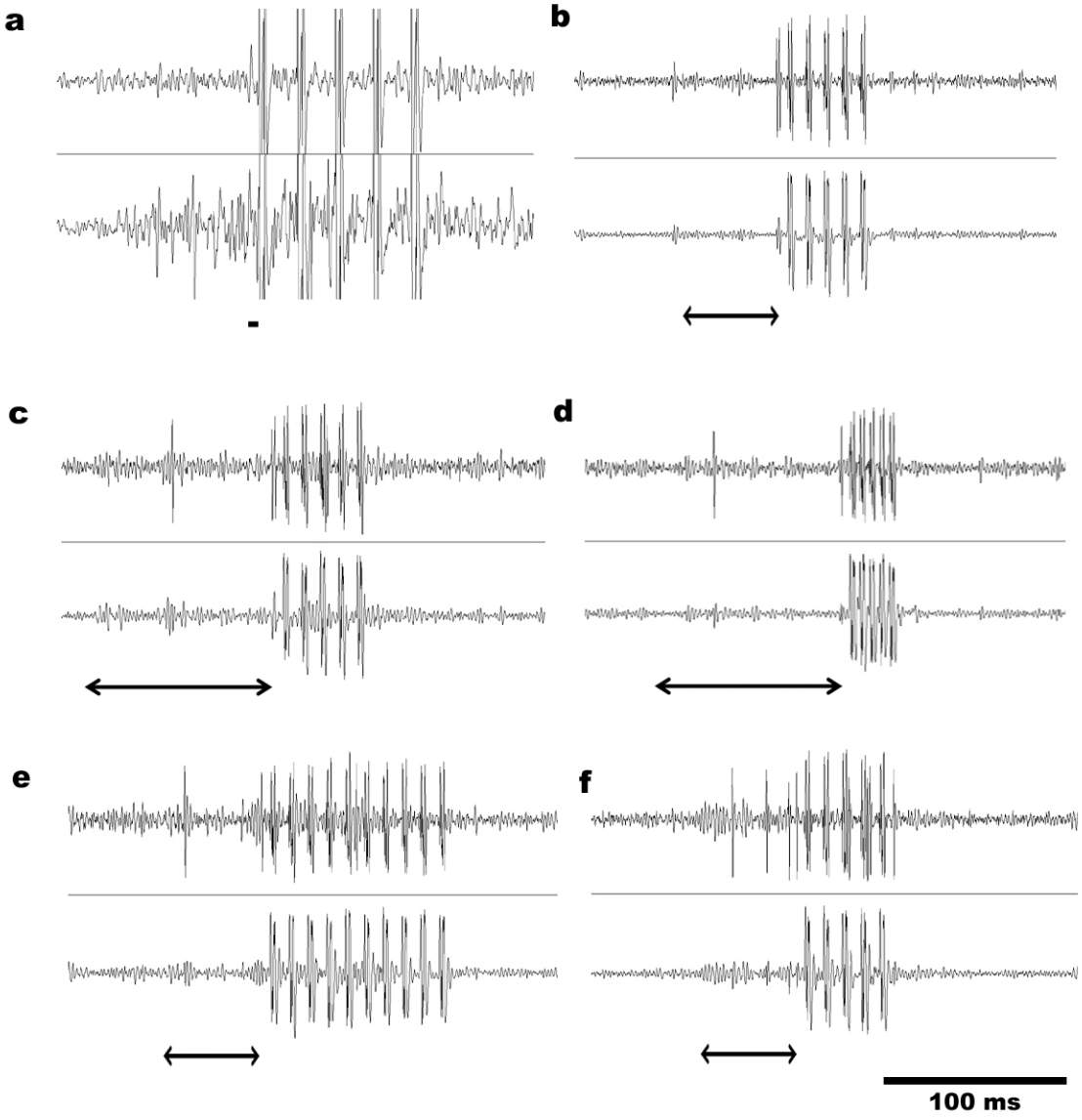

Figure 4.33 Different stimulation patterns triggered by neural signals from the primary motor cortex. a. stimulation (40 Hz, 5 pulses) was triggered by 1 spike. **b.** stimulation (91 Hz, 5 pulses) was triggered by 2 spikes occurred within the time window of 50 ms as double arrow indicates , the interval between 2 spikes is 1.6 ms. **c.** stimulation (91 Hz, 5 pulses) was triggered by 2 spikes occurred within 100 ms, Δ=63.4 ms. **d.** stimulation (167 Hz, 5 pulses) was triggered by 2 spikes occurred within 100 ms, Δ=79.3 ms. **e.** stimulation (91 Hz, 10 pulses) was triggered by 2 spikes occurred within 50 ms, Δ=46.4 ms. **f.** stimulation (91 Hz, 5 pulses) was triggered by 4 spikes occurred within 50 ms, Δ=39.1 ms.

4.3.3.2 Neuronal signals transmitted to the computer wirelessly

At the speed of 5.6 cm/s, the extracellular signal of the hindlimb region in M1 showed rhythmic activity with high-amplitude action potential. The rhythmic discharge of M1 synchronized with the EMG of the left forelimb, with a time lag of approximately 150 ms (Figure 4.35 a). Once the stimulator switch was turned on, the extracellular cortical signal triggered a 5-pulse train when the signal reached the triggering threshold (0.2 V) (Figure 4.35 b-c). During a period of 41 s running, the stimulator switch was turned off in the first ten seconds and the stimulator switch was turned on at the eleventh second, as indicated by a flash (Figure 4.35 d). Every hindlimb movement followed by 32 steps was detected during the 31.0 s of walking. Three odd electrical stimulations were spotted in the middle of the walking period, as indicated by a single arrow (Figure 4.35 d). These odd steps can be observed from the artifacts of electrical stimulation on extracellular and EMG signals clearly. The left hindlimb showed a good temporal coordination with the left forelimb. Response of the left hindlimb commenced behind the stepping of the left forelimb, which was observed in the stance phase of left forelimb (Figure 4.34 b and d) rather than in the swing phase (Figure 4.34 a and c). A movie demonstrating the hindlimb movement of the same subject is attached in Video 4-8 (29 s). This video shows 3 stages: stationary stand for 5 second, running without stimulation for 10 second and running with stimulation for 14 second. Just during the stage of running with stimulation, there is regular stimulated hindlimb movement. The movement is representative response achieved in the guinea pigs with wireless Motolink, when they walked on the treadmill by forelimb and supported by harness at speed of 5.6 cm/s.

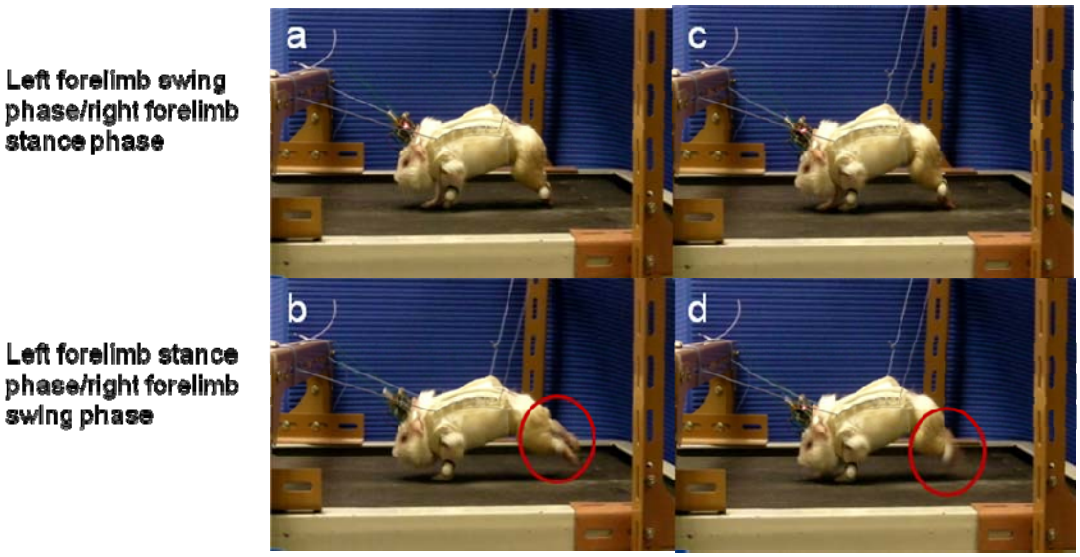

Figure 4.34 Movement of the left hindlimb was observed in the left forelimb stance phase. a-b. One step circle of the left forelimb. **c-d.** Another step circle of the left forelimb. **b, d.** Movement of the left hindlimb commenced in the stance phase left forelimb, which is marked by red circles.

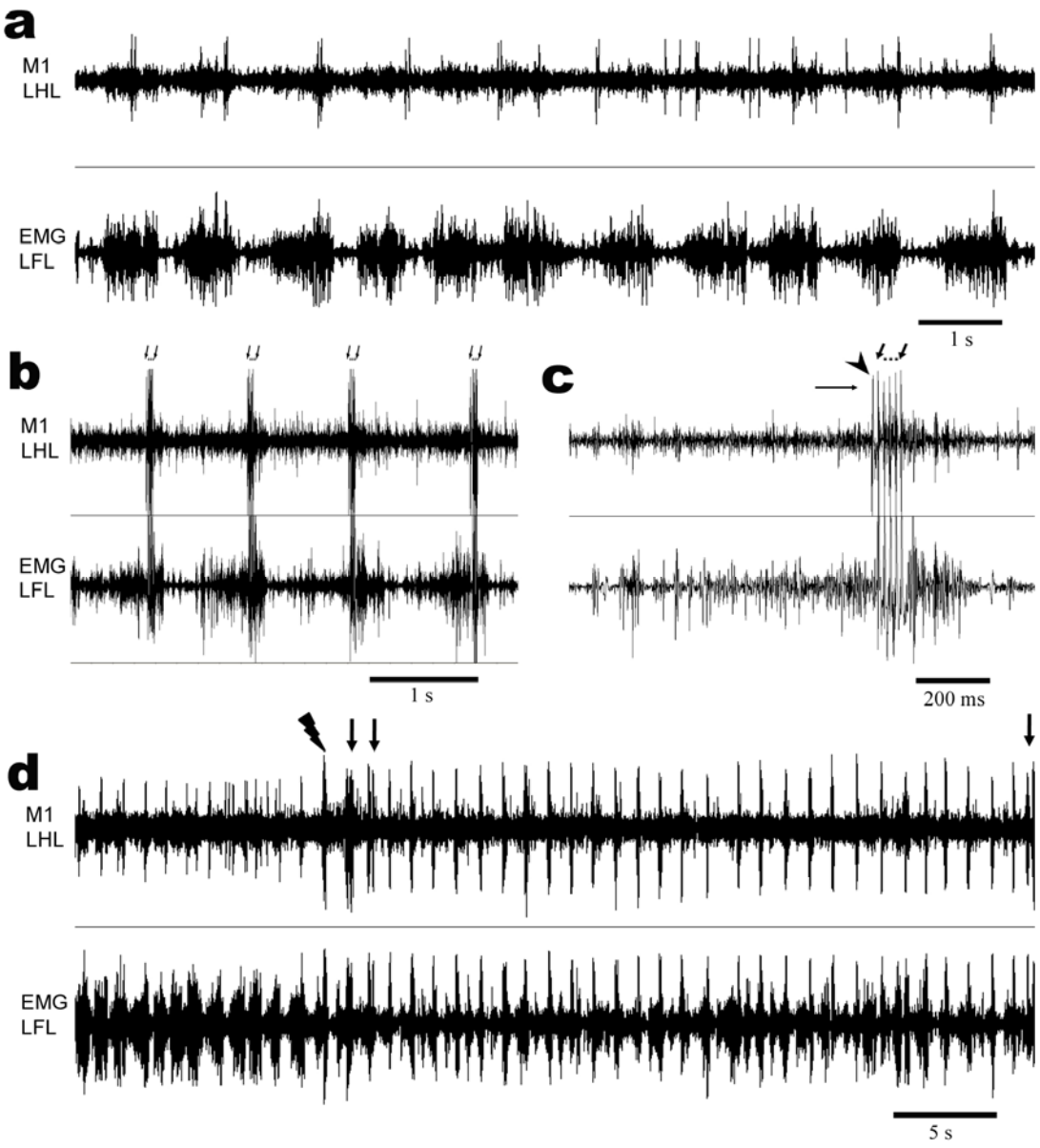

Figure 4.35 Neuronal ensembles and EMG recorded by Motolink via wireless data transfer when animal (subject #5) was walking on the treadmill at a speed of 5.6 cm/s. a. Extracellular neuronal signal (upper panel) from the left hindlimb (LHL) region of the right motor cortex (M1) and EMG signal (lower panel) from the muscle group for extension in the left forelimb (LFL) were recorded while the animal was walking on the treadmill by its forelimb at a speed of 5.6 cm/s. Signals were recorded when the stimulator was switched off. **b.** Signals from the same electrodes (M1 and EMG) recorded from the same subject when the stimulator was switched on. Electrical stimulation of the LHL generated artifacts in the recording electrodes, as marked with oblique arrows. **c.** The trigger threshold of the neuronal signal was set at 0.2 V and is marked with a horizontal arrow. The trigger signal is marked by an arrow head. The

time interval between the trigger signal and the electrical stimulation was set at 5 ms. **d.** A 41 s-episode of recordings of the neuronal and EMG signals was sampled when the subject was walking on the treadmill at 5.6 cm/s. In the first ten seconds the stimulator switch was turned off and no triggered response of hindlimb was observed. From the eleventh second to the end, the stimulator switch was turned on and stimulated response of hindlimb was recorded. The start of stimulation is indicted by a flash symbol.

4.4 Discussion

4.4.1 Mapping of the hindlimb area of the primary motor cortex

Gluteus superficialis, biceps femoris, semitendinosus, and tibialis anterior are four superficial muscles of the hindlimb and the usually used muscles for EMG recording (Widajewicz, Kably et al. 1994; Bretzner and Drew 2005). We inserted recording electrodes into these four muscles. Most EMG activity of these four muscles was activated by upper-threshold stimulation. In some case, it required high current, such as 250 μA to innervate the movement of hindlimb. This might due to the low impedance of some stimulation electrodes (10-30 kΩ). The rhythmic contraction of forelimb or hindlimb caused by given ISMS was not distinguishable when the rhythmic jaw movement was elicited by excess electrical stimulation in some situation ((Goldberg and Chandler 1981; Nakamura, Muramatsu et al. 1990; Katayama, Kohase et al. 1993).

4.4.2 Development of Motolink

From the beginning to the present, the development of Motolink experienced stages of wired transfer and wireless transfer to computer. Recording with wires limited the activities of animals and received more interference from those connection wires. Technique of telemetry provides larger scope of activities either on the treadmill or freely moving, as well as lighter weight and smaller size. Wireless recording is a popular trend of chronic experiment and application of neuroprostheses for clinical trials. For long-term application, it will require telemetry for the convenience of patient

movement and avoid the percutaneous connections which provide routes for infection, the same as the intracortical electrodes as for ECoG electrodes (Wolpaw, Loeb et al. 2006). At present, the distance of RF transfer limits to 1 meter. Large range is required for behaving freely in future application.

Intraspinal functional electrical stimulation is an approach of stimulating the motor neurons and interneurons in the spinal cord rather than stimulating muscle units. Compared to muscles, neuron in the central neural system is less easy to become fatigue and no response after long time stimulation. The present stimulation pattern was voltage stimulation. However, for chronic experiments, current stimulation has obvious advantage over voltage stimulation. It is because in chronic experiments the impedance of electrodes will decrease gradually as the tip of electrode is surrounded by glia cells several days after surgery. Current stimulation will keep relatively stable output during stimulation delivery even though the impedance of electrodes varied in different days after surgery and constant hindlimb response. However, output of voltage stimulation can be influenced by the decrease of electrodes impedance.

4.4.3 Reconnection of upper motor neurons and lower motor neurons by Motolink

For the first time, we have successfully linked UMNs with LMNs using an electronic pathway in animals with SCI. To study the behavioral effects of spinal cord stimulation, we implanted an electrode array into the hindlimb region of the right motor cortex. Only one electrode was used to pick up neuronal signals from M1. After amplification, the neuronal signals triggered stimulation of LMNs. Use of multiple

choices by four electrodes increased the success rate in reading neuronal activity of the motor neurons. The recording site was roughly mapped before the recording electrode array was implanted. The rhythmic firing of the recorded neurons was a reflection of motor cortex when the animal was placed on a moving treadmill. Histological results showed that the electrodes were implanted in the UMN layer, layer V, of M1. The time lag between the movement of stimulated left hindlimb and the intact left forelimb was a further proof that the recording was from the hindlimb region of M1. The time lag shortened when the speed of the treadmill increased. For the behavioral experiments, we implanted seven stimulation electrodes in the spinal cord, targeting the hindlimb region. The electrode that triggered the maximal movement of the hindlimb was selected for stimulation. The presence of multiple stimulation electrodes increased the chance that one electrode would effectively stimulate the hindlimb muscles.

The parallel and sequential commands from the UMNs are conveyed to the LMNs through the corticospinal tract. These signals are interpreted by LMNs, which, in turn, send signals to target muscles that produce muscle contraction. The integration of UMN commands by LMNs is not one-to-one and not linear. Therefore, functional electronic bridges must be capable of non-linear conversions in order to be a useful therapy for human SCI.

Our results show the time gap (100~200 ms) between the cortical action potentials and EMG which demonstrates the sequence of forelimb and hindlimb movement in gait of step. Following the forelimb stepping, the intent to move its hindlimb is originate in the primary motor cortex. Although these intent signals cannot be deliver physically to lower motoneurons in subjects with injured spinal cord, we can easily record these activities in M1 hindlimb area.

We employed different type of animal as subjects in acute experiment and chronic experiment. In anaesthetized situation, we used rats as acute spinal cord injury models. In chronic experiments we applied guinea pigs as subjects since the brain and the spinal cord of guinea pig are larger than those of rat. Hence it is easier for the implantation of electrodes during surgery preparation than in rats. Most importantly, guinea pig has a good nature and is less possible to hurt experimenter when it is trained to walk on the treadmill by harness, but rats tends to be irritated in the same situation.

4.4.4 Chronic implantation of electrode array

Since the materials of recording electrode applied in our experiments were either tungsten insulated with Teflon or platinum insulated with parylene, they had good bio compatibility and the minimum damage to the brain tissue. At present, the earphone wires are used as stimulating electrode. It is soft and has good flexibility but the bio compatibility is not as good as commercial stainless steel. This experiment need to insert electrode array into the brain which did make acute and chronic damage to the brain tissue, which is an unsolved issue of chronic experiments (Grill and Mortimer 2000; Polikov, Tresco et al. 2005). Many chronic experiments have the problem of lose the ability to record spike activity in short period after electrode implantation (Grill and Mortimer 2000; Vetter, Williams et al. 2004). At present, studies on Motolink have to been limited within one month because almost no meaningful signals can be collected from the electrodes after 4 weeks. However, recent encouraging results on reducing the reaction in implanted arrays and promotion of long-term

functional stability raise hope for using neural prosthetic devices (Serruya, Hatsopoulos et al. 2002; Shain, Spataro et al. 2003; Retterer, Smith et al. 2004).

4.5 Summary

For the first time, we have successfully linked UMNs with LMNs using an electronic pathway in animals with SCI. We recorded that twitching of left hindlimb was subsequent to each step of left forelimb as guinea pig walking on the treadmill. Time lag between the EMG activity of forelimb and hindlimb was statistically different as the speed of treadmill changed. As shown in the corresponding videos, mostly every step of forelimb is followed by one response in lower limb. At present, tissue damage and recording stability are still unsolved problems of studies on chronic recording. Telemetry for to data report to computer wirelessly has been achieved in this study, which is essential to long-term application and clinical usage.

CHAPTER 5 CONCLUSIONS AND SUGGESTIONS FOR FUTURE REASERCH

5.1 Conclusions of This Study

Currently Motolink is a miniaturized neuroprostheses with low-noise, high-input-impedance, high-gain amplifiers, and programmed interface. We have proved good performance of animal with spinal cord injury using Motolink. The real-time cortical signal can be acquired to trigger stimulation pulses, and then delivered to the motor neurons in the spinal cord to induce locomotion of hindlimb.

Our findings showed that neural response of VC, AC or MGB to stimuli of light, sound or electrical current were exactly acquired by "Motolink" as the frequency of stimuli changed in acute experiments. Twitching of lower limb was observed every time when signals were acquired from different areas to activate spinal motoneurons. In chronic experiments, we recorded twitching of left hindlimb which was subsequent to steps of left forelimb as guinea pig walking on treadmill by its forelimb. Time lag between the onset of EMG activity of forelimb and spikes of M1HL was statistically different as the speed of treadmill changed. As shown in the corresponding videos, mostly every step of forelimb is followed by one response in lower limb, but sometimes followed by none or two, even more.

We successfully reconnected the upper motor neuron in the primary motor cortex with the lower motor neuron in the spinal cord below the lesion in animals with spinal cord injury, which electronically bypasses the spinal cord injury lesion. Our findings

show artificially electronic pathway of Motolink is possibly an alternative strategy for the recovery of functional locomotion of hind limbs in patients with spinal cord injury.

5.2 Suggestions for Future Research

We have succeeded in reconnecting UMNs with LMNs using one channel. This was an important first step in building electronic bridge to restore movement after SCI. Further goal of our experiment is, by multichannel Motolink, to analyze cortical neural ensembles in real time and transfer signals to a group of microstimulation, which can activate the motor pool in the spinal cord below the lesion, hence to reanimate paralyzed hindlimbs to produce smoothly and graceful movements.

There are many technical issues required to be improved for the real functional recovery from paralysis. The second step has been set to develop the multiple channels circuit to connect UMNs and LMNs in parallel. A third step will be to enable non-linear conversion learning between the input and output functions of the LMNs. A multichannel, non-linear circuit may enable paralyzed patients to regain the ability to move and walk.

Firstly, multichannel amplifier with multichannel wireless transmitter and receiver, together with multichannel stimulator are required to be developed. We need to record parallel cortical signals from bilateral brain by multichannel and transform them to a group of stimulation pulses, which to be convey to a group of motoneurons in different level of the lumbar spinal cord. The number of electrodes in the electrode array of motor cortex recording is at least expanded to 4, which can acquire more accurate signals in target area of the primary motor cortex and produce basic functional locomotion.

Secondly, in the future we are going to explore neural networks control algorithm for better decoding of neural activities. It will enable non-linear conversion learning

between the input and output functions of the LMNs. Effective microstimulation program is required to be developed based on cortical commands for facilitation of controlling the spinal cord to produce hindlimb stepping naturally and smoothly in animals with spinal cord injury. Moreover, skill of inserting the stimulation electrodes to the ventral horn of the lumbosacral enlargement should be more accurate and precise, which can mainly target those motoneurons associated with muscles of extensor and flexor in knee and ankle.

With respect to the subjects, if it was approved by the animal ethical committee, adult cats could be used as subjects in the future for its spinal cord is relatively large. We can directly apply previous findings on the locomotion-related network in the lumbosacral enlargement of the adult spinal cat (Mushahwar and Horch 1998; Mushahwar, Collins et al. 2000; Mushahwar and Horch 2000; Mushahwar and Horch 2000; Mushahwar, Gillard et al. 2002; Mushahwar, Aoyagi et al. 2004). Since the bio compatibility of earphone wire is not reliable, we are considering of applying Teflon insulated stainless steel in the coming experiments. Stainless steel microwires (50 μm diameter, 30 mm tip exposure, 10 to 30 kΩ impedance, Teflon insulated, A-M systems, Inc., WA, USA) are the preferred material of making electrodes of intraspinal microstimulation in vivo.

Sensory feedback control and will be concerned for real functional movement in clinical application. Since in patient with spinal cord injury complete separation of the spinal cord is very rare (Schwab 2002), the remain neuron and axon still function. Therefore, the recovery of remaining structure should be considered. Combination of several therapies is a trend in the treatment of spinal cord injury.

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