

Rebuilding motor function of the spinal cord based on functional electrical stimulation

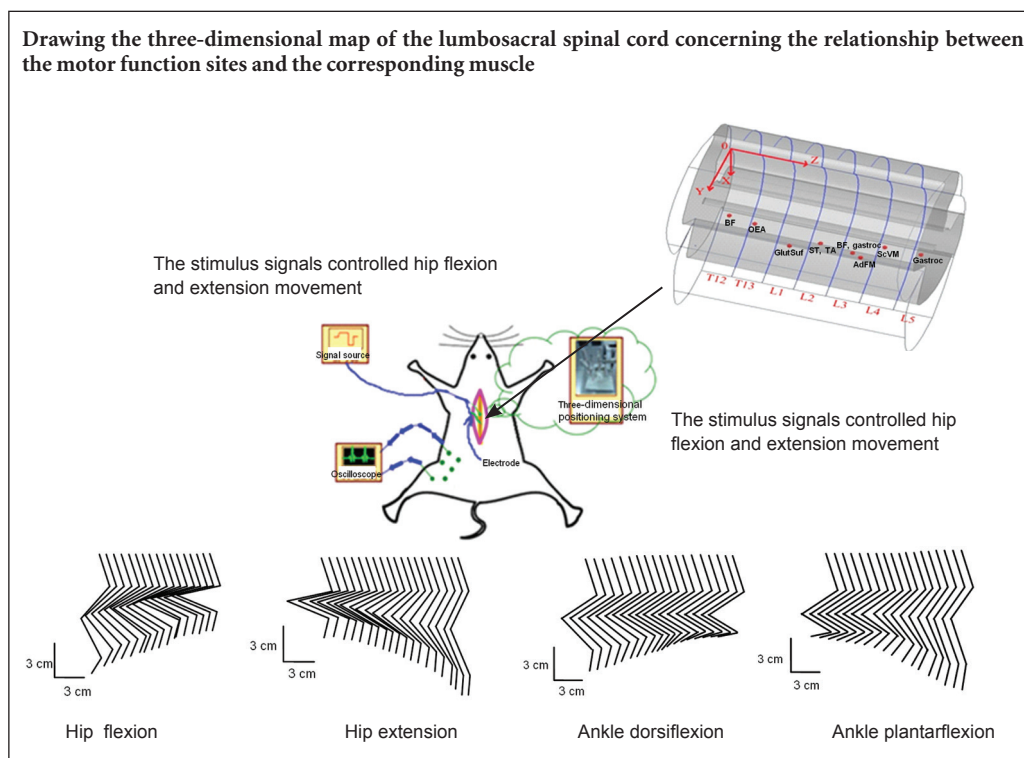
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Graphical Abstract



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Abstract

Rebuilding the damaged motor function caused by spinal cord injury is one of the most serious challenges in clinical neuroscience. The function of the neural pathway under the damaged sites can be rebuilt using functional electrical stimulation technology. In this study, the locations of motor function sites in the lumbosacral spinal cord were determined with functional electrical stimulation technology. A three-dimensional map of the lumbosacral spinal cord comprising the relationship between the motor function sites and the corresponding muscle was drawn. Based on the individual experimental parameters and normalized coordinates of the motor function sites, the motor function sites that control a certain muscle were calculated. Phasing pulse sequences were delivered to the determined motor function sites in the spinal cord and hip extension, hip flexion, ankle plantarflexion, and ankle dorsiflexion movements were successfully achieved. The results show that the map of the spinal cord motor function sites was valid. This map can provide guidance for the selection of electrical stimulation sites during the rebuilding of motor function after spinal cord injury.

Key Words: nerve regeneration; spinal cord injury; functional electrical stimulation; rebuilding motor function; movement control; spinal cord; lumbosacral spinal cord; motor function sites; hip extension movement; hip flexion movement; ankle plantarflexion; ankle dorsiflexion; neural regeneration

Introduction

Spinal cord injury (SCI) can impair the motor function of patients to different degrees, and directly impacts the patients' lifestyle and quality of life. Thus, there is an urgent need to explore effective ways to restore motor function of patients with SCI (Griffin et al., 2009; Musienko et al., 2009; Borton et al., 2014). Functional electrical stimulation (FES) has been widely used as a method to artificially activate the motor system after central nervous system injury (Dejan, 2014). So far, FES has been used in clinical trials successfully (Hart et al., 1998; Burridge et al., 2007). It has been used to control the arm and hand or to promote standing, balance, posture and gait training in the lower limbs (Pfurtscheller et al., 2003; Kim et al., 2008; Braz et al., 2009). Also, FES induces limb movements and promotes the motor function recovery of animals with SCI (Mushahwar et al., 2002, 2004; Bamford et al., 2005; Mondello et al., 2014). Because limb movements cannot occur with only a single muscle contraction, a natural motor behavior is formed by the combination of muscle synergies (d'Avella et al., 2003; Drew et al., 2008; Yu et al., 2009). Electrical microstimulation research has provided the most direct and powerful evidence that the nervous system encodes motor primitives, whether the corresponding relationship exists in the spinal cord or cortex (Tresch et al., 1999; Graziano et al., 2002; Haiss et al., 2005; Zimmermann et al., 2011). There is no suitable spinal cord motor function map that is used for FES, so the positions of many implanted microelectrodes have been determined by trial and error of a specific movement (Lemay et al., 2009). Therefore, FES technology has been used to determine the corresponding relationships between the motor function sites in the spinal cord and the muscles that control the hindlimb movements. On this basis, rebuilding hindlimb motor function with FES was studied.

Materials and Methods

Animals

Seven adult specific-pathogen-free male Sprague-Dawley rats (~250 g body weight) were provided by the Experimental Animal Center of Nantong University in China (animal license No. SCXK (Su) 2014-0001). After intraperitoneal anesthesia with chloral hydrate (4 mL/kg), the rats' backs and left hindlimbs were shaved and laminectomies were performed at the T₁₂-L₅ vertebral segments (the distance of the spinous process of the thoracic vertebra is shortest between the ninth, the tenth and the eleventh). The spinous process trended to caudal-ward above the ninth thoracic vertebra, the tenth was in the neutral position, and the spinous process trended to head-ward below the eleventh (Shang et al., 2013). The reference electrode was implanted in the adjacent paraspinal muscle. Bipolar needle electrodes were implanted in the eight muscles of the left hindlimb: biceps femoris, obliquus externus abdominis, gluteus superficialis, semitendinosus, tibialis anterior, adductor femoris magnus, sacrocaudoccygeus ventralis medialis, and gastrocnemius, which were

used to confirm whether the relevant muscle contraction was induced by the electrical stimulation. Rats were placed on a heating pad to maintain body temperature at approximately 37°C. All surgical procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the local Animal Studies Committee.

Stimulation electrode and stimulation parameters

Monopolar stimulation with a tungsten needle microelectrode (WE30030.5A3; MicroProbes for Life Science, Gaithersburg, MD, USA) with a diameter of 2–3 μm and 30–70 μm exposed at the tip was used to stimulate the spinal cord. Monophasic, cathodic, voltage-controlled (400–800 mV) pulse trains generated by a stimulator (master 9, A.M.P.I., Jerusalem, Israel) were applied in the experiment. Each train consisted of 40 negative pulses, and each pulse lasted 200 μs with a frequency of 33 Hz. The pulse trains were repeated every 4 seconds.

Experimental procedure

The experimental procedure of positioning the spinal cord motor function sites of the rat *via* FES is shown in **Figure 1**. Each rat was laid down on the Stereotaxic System (51750; Stereotaxic Instrument, Stoelting, IL, USA) and the spinal cord was fixed with the rat spinal cord adaptor (51695, Stereotaxic Instrument). FES was used in the exposed spinal cord, and the spinal cord locations that could induce a certain muscle contraction were determined. The locations of the stimulation sites were described as the positions in the corresponding vertebral segment, denoted as (X, Y, Z). X is the distance that the stimulation site deviated from the posterior median sulcus of the spinal cord (left '-', right '+'). Y is the depth of the stimulation site (distance from the highest location of the spinal cord surface). Z is the distance from the stimulation site to the rostral margin of the corresponding vertebral segment. Electrical stimulation was delivered to the stimulation electrode, which was moved in the cross-sectional and the rostrocaudal directions of the spinal cord at certain intervals. Stimulation intensity was regulated and the different locations that could induce hindlimb muscle contraction were determined. The region that induced a certain muscle contraction was determined. The thresholds of the different stimulation sites in the region were compared, and the stimulation site with the lowest threshold was determined as the optimal spinal cord stimulation site (the rats were anesthetized deeply). The optimal stimulation sites that control different muscle contraction were normalized. The stimulation sites of other rats were calculated according to the processed data. Phasing pulse sequences were delivered to the different motor function sites in the spinal cord to induce hindlimb coordinated movements. Electromyography (EMG) signals were recorded (2 kHz), amplified and filtered (30–1,000 Hz).

Normalized coordinate description of the stimulation sites

To describe the motor function locations in the spinal cord accurately, the coordinates that control different muscle con-

Table 1 Normalized coordinates of motor function sites in the spinal cord

Spine	Muscle	X	Y	Z	Threshold (mV)
T ₁₂	Biceps femoris	0.3550±0.0046	0.3710±0.0038	0.6350±0.0180	720
T ₁₃	Obliquus externus abdominis	0.4320±0.0003	0.3560±0.0054	0.4450±0.0170	763
L ₁	Glutaeus superficialis	0.4950±0.0109	0.4620±0.0034	0.7370±0.0009	700
L ₂	Semitendinosus, tibialis anterior	0.5540±0.0054	0.3780±0.0044	0.5380±0.0115	600
L ₃	Biceps femoris, gastrocnemius	0.6190±0.0154	0.3690±0.0022	0.5080±0.0064	575
L ₃	Adductor femoris magnus	0.6780±0.0059	0.3540±0.0033	0.7460±0.0076	600
L ₄	Sacrocoecygeus ventralis medialis	0.3410±0.0056	0.3690±0.0018	0.5060±0.0079	575
L ₅	Gastrocnemius	0.3850±0.0079	0.3550±0.0014	0.5520±0.0180	475

Data are normalized and the coordinates (X, Y, Z) are described as the mean ± SD (n = 6).

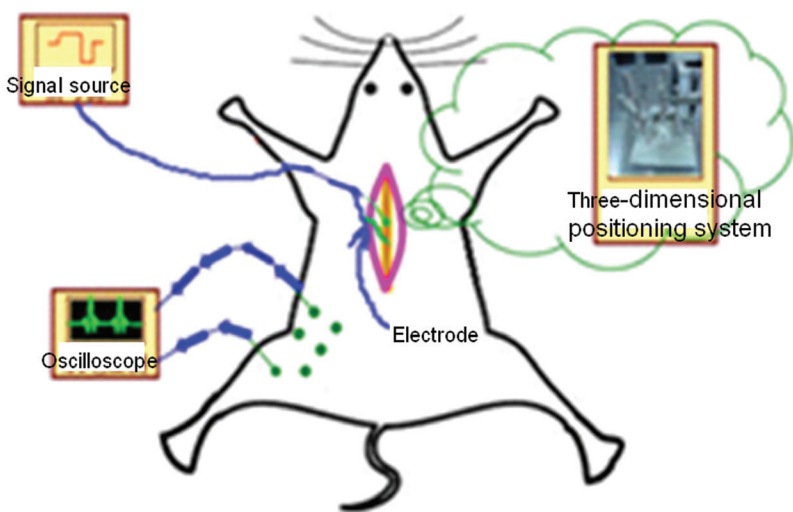


Figure 1 Experimental schematic indicating the positioning of the motor function sites in the spinal cord of the rat via functional electrical stimulation.

The stimulation electrode was implanted in the spinal cord, marked yellow. Electromyography was acquired with an oscilloscope when the stimulation signal was delivered to the motor function sites via the stimulation electrode.

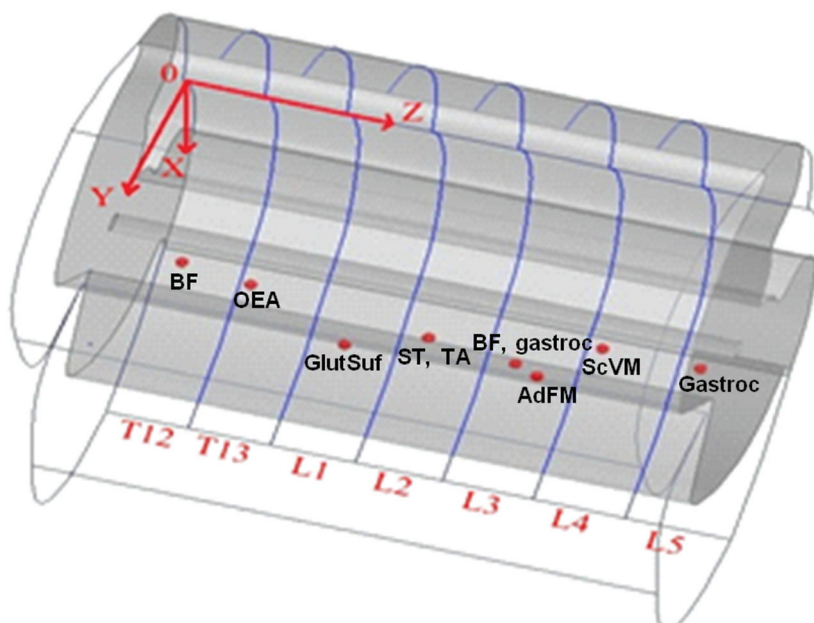


Figure 2 The three-dimensional map depicts the spinal cord motor function sites.

The coordinates (X, Y, Z) about the stimulation sites that can induce various muscle contraction of the hindlimb, including biceps femoris (BF), obliquus externus abdominis (OEA), glutaeus superficialis (GlutSuf), semitendinosus (ST), tibialis anterior (TA), adductor femoris magnus (AdFM), sacrocoecygeus ventralis medialis (ScVM) and gastrocnemius (gastroc).

traction were normalized. Through analysis and verification of the two normalization methods relative to the vertebral segment or the total length of spinal cord (Huang et al., 2012), the normalization method relative to the vertebral segment was adopted to describe the motor function sites.

Coordinate X was normalized by the half transverse diameter of the spinal cord $D/2$ (D was the transverse diameter of the spinal cord). Coordinate Y was normalized by the transverse diameter (D). Z was normalized by the length L of the vertebral segment in the rostrocaudal direction.

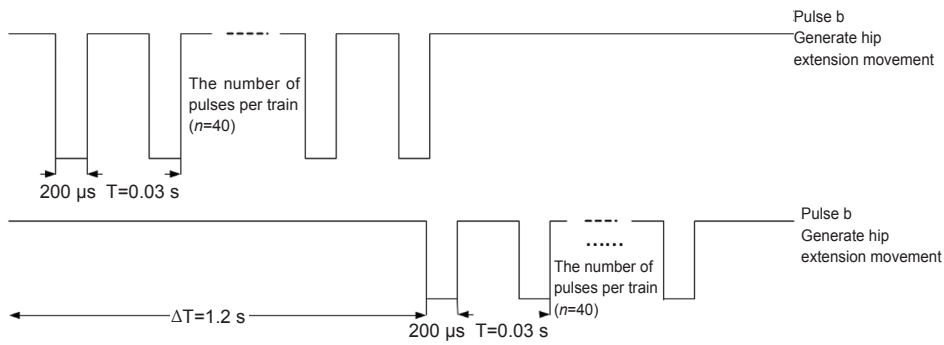


Figure 3 Stimulus signals controlling hip flexion and extension movement.

Hip flexion movement was generated with pulse train a and hip extension movement was generated with pulse train b. The duration of the pulse was 200 μs. The frequency of the stimulus signal was 33 Hz. The number of pulses per train was 40. When pulse train a finished, pulse train b began. T: Time; s: second.

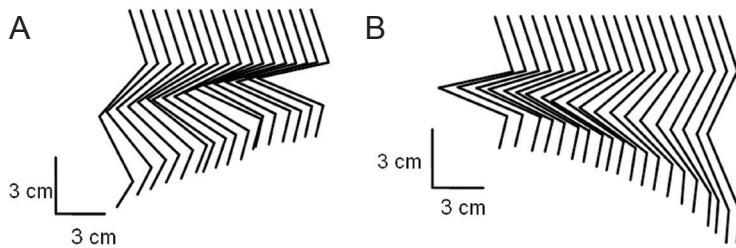


Figure 4 Hip extension and flexion movements based on functional electrical stimulation.

(A) Hip flexion movement occurred when electrical stimulation was delivered at coordinates (X, Y, Z) = 1.0, 1.3, 3.1 mm; (B) hip extension movement occurred when electrical stimulation was delivered at coordinates (X, Y, Z) = 0.8, 1.5, 3.6 mm.

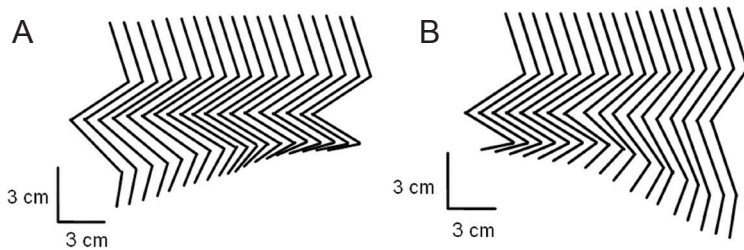


Figure 5 Ankle plantarflexion and dorsiflexion movements based on functional electrical stimulation.

(A) Ankle dorsiflexion movement occurred when electrical stimulation was delivered at coordinates (X, Y, Z) = 1.0, 1.3, 3.1 mm; (B) ankle plantarflexion movement occurred when electrical stimulation was delivered at coordinates (X, Y, Z) = 1.1, 1.2, 4.6 mm.

Results

The map about motor function sites

Following the above experimental procedure, the experiment of positioning the motor function sites with a frequency of 1 Hz was conducted on the six rats. The coordinates of the spinal cord motor function sites that induce muscle contraction were normalized and recorded as (X, Y, Z). The data on the normalized coordinates are shown in **Table 1**.

According to the experimental data in **Table 1**, based on the average diameter of the spinal cord and the average length of each rat's spine, a three-dimensional map of the spinal cord stimulation sites that can induce hindlimb muscle contraction is shown in **Figure 2**. The spinal segments corresponding to the T₁₂-L₅ vertebral segments are depicted in **Figure 2**. The vertebral segments were separated by blue lines. The locations of the optimal spinal cord motor function sites that can induce biceps femoris, obliquus externus abdominis, gluteus superficialis, semitendinosus, tibialis anterior, adductor femoris magnus, sacrococcygeus ventralis medialis, and gastrocnemius to produce muscle contraction are shown with red dots on the map. Determining the corresponding relationship between the motor function sites and the muscles can provide guidance for the next few experiments to achieve hip flexion, hip extension, ankle plantar-

flexion, and dorsiflexion movements.

Movement control with FES

Lumbosacral spinal cord can induce simple muscle contraction with low-frequency electrical stimulation, but coordinated movements can only be induced at a higher frequency (Lavrov et al., 2015). According to the data in **Table 1**, the transverse diameter and the length of the vertebral segments of the 7th rat, the positions that control the hip flexion, hip extension, ankle plantarflexion and dorsiflexion could be calculated.

Hip movement control with FES

The location calculated with related data that control the hip flexion of the 7th rat is located in the spinal cord corresponding to the first lumbar vertebra, near coordinates (X, Y, Z) = 0.8, 1.5, 3.6 mm. The location that controls the hip flexion is located in the third lumbar vertebra, near coordinates (X, Y, Z) = 1.0, 1.3, 3.1 mm.

The extension and flexion movements of the hip joint are controlled by two phasing pulse sequences as shown in **Figure 3**. Hip flexion movement was generated with pulse train a, and hip extension movement was generated with pulse train b. Stimulation signals a and b were delivered to two tungsten electrodes that were located in the corresponding

spinal cord motor function sites. The threshold voltage of the hip flexion movement was 800 mV and the threshold voltage of the hip extension movement was 250 mV.

The kinematics of the hip movements of the left hindlimb are displayed in **Figure 4**. As can be seen in the figure, accompanying the hip flexion and extension movements, slight movements of other joints can be evoked within these locations.

Ankle movement control with FES

The experimental procedure of ankle movement control was consistent with the experimental procedure of hip movement control. The stimulus signals shown in **Figure 3** were still used, but the difference was that ankle plantarflexion movement was generated with pulse train a and ankle dorsiflexion movement was generated with pulse train b. The location that controls the ankle dorsiflexion of the 7th rat is located in the spinal cord corresponding to the second lumbar vertebra, near coordinates $(X, Y, Z) = 0.9, 1.3, 3.3$ mm. The location that controls the ankle plantarflexion is located in the third lumbar vertebra, near coordinates $(X, Y, Z) = 1.1, 1.2, 4.6$ mm. The kinematics of the ankle movements of the left hindlimb is shown in **Figure 5**. Accompanying the ankle dorsiflexion and plantarflexion movements, slight movements of the knee joint can also be seen in **Figure 5**.

Discussion

In this study, FES technology was used to position the motor function sites in the spinal cord that can induce hindlimb muscle contraction. The normalized locations of motor function sites were summarized and a three-dimensional map of the relationship between the motor function sites and the corresponding muscles was drawn. All this can be the basis of further study using functional reconstruction experiments in SCI rats *via* electrical means. Phasing pulse sequences were delivered to the corresponding motor function sites in the spinal cord, and hip extension, hip flexion, ankle plantarflexion, and dorsiflexion movements were achieved successfully. Thus, fluid movements of the hindlimb can be induced using phasing pulse sequences that are delivered to a small amount of electrodes implanted in the corresponding functional sites of the lumbosacral spinal cord.

Some scholars have drawn maps to describe the output response of electrical stimulation at the ventral horn of the spinal cord or the motoneuron distribution in the lumbosacral spinal cord (Mushahwar and Horch et al., 1997, 1998). However, so far, there is no description of the corresponding relationship between the optimal motor function sites and the corresponding muscles. Independent control of all the joints and muscles can be achieved with the above corresponding relationship and the parameters of the animal model. In the experiment, a simple muscle contraction response could only be seen with a stimulus frequency of 1 Hz, while a variety of hindlimb movements could be induced with frequency ~30 Hz. This is because

of the varieties of motoneurons that are compactly distributed in the lumbosacral spinal cord (Vanderhorst et al., 1997; Yakovenko et al., 2002). When electrical stimulation was delivered to the tip of the microelectrode implanted in the lumbosacral spinal cord, the motoneurons located at the tip of and around the microelectrode were activated simultaneously, and the motor output was expressed as the co-effect of multiple muscle contractions. In addition, because the number of interneurons is seven times that of the motoneurons within these stimulated regions (Henneman, 1980), the flexion and extension movements of the joints and other coordinated movements might arise from the activation of neuronal circuitry spanning the lumbosacral, and indirectly activated motoneurons. It is reported that rhythmic activities can be evoked with tonic intraspinal microstimulation (Guevremont et al., 2006; Lavrov et al., 2015). However, alternating, locomotor-like stepping was not found in our experiments, which may be because of suppression from the higher central nervous system. More experiments need to be conducted on spinal rats to investigate the mechanism of the locomotion generated by FES in the spinal cord.

As a form of FES, intraspinal microstimulation shows promise in clinical applications, and indices better selective activation of locomotor-related networks in the spinal cord than does epidural electrical stimulation (Musbahwar et al., 1998, 2000). More importantly, intraspinal microstimulation can activate propriospinal pathways located superficially, making it more feasible in clinical applications (Lavrov et al., 2015). Further, intraspinal microstimulation can evoke natural movements of the hindlimb by recruiting muscles in a synergistic way, and preferentially recruits fatigue-resistant muscle fibers, so the issue of fatigue is reduced significantly during electrical stimulation (Mushahwar and Horch et al., 2000; Bamford et al., 2011; Verhaagen et al., 2012). Because of the complexity of the neural networks in the lumbosacral spinal cord, further investigations will be needed into the distribution of motoneurons, interneurons and axons in the lumbosacral spinal cord, which requires more electrophysiological studies (Mushahwar et al., 2002). Other methods can be combined to promote the recovery of hindlimb motor function in follow-up experiments, such as pharmacological therapy and treadmill training. Research on this topic will provide guidance on the subsequent experiment addressing the recovery of motor function.

Author contributions: *The overall design of the experiment was agreed by all authors after extensive discussions. XYX designed the study. WH performed the study. YC drew the diagrams. WH and WD analyzed the experimental data and wrote the paper. All authors approved the final version of the paper.*

Conflicts of interest: *None declared.*

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