Effect of Treadmill Training Protocols on Locomotion Recovery in Spinalized Rats

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Abstract

Both treadmill training and epidural stimulation can help to reactivate the central pattern generator (CPG) in the spinal cord after a spinal cord injury. However, designing an appropriate training approach and a stimulation profile is still a controversial issue. Since the spinal afferent signals are the input signals of CPG in the spinal cord, it can be concluded that the number of input afferent signals can affect the quality of movement recovery, such a phenomenon is in accordance with Hebbian theory. Therefore, at first in this paper, through some simulation studies on a model of CPGs, the effective influence of increasing the afferent input weight on activating CPG model was certified. Then, the performance of two different types of treadmill training along with epidural stimulation was compared. The numbers of spinal afferents involved during each designed training approach were different. Experiments were conducted on two groups of spinalized rats. Three quantized integer qualitative measures, with 0–2 scales, were envisioned to evaluate the performance of training protocols. According to the experimental results, the assigned scales to the rats using the training approach involving more afferents, the rats have been creeping on a treadmill, was 2. Also, the assigned scales to the rats using the training approach involving less afferents, the rats have been performing bipedal locomotion, was 0 or 1. Such experimental results coincide with achieved simulation results elucidating the effect of increasing the afferent input weights on activating CPG model.

Keywords: *Animals, central pattern generators, dinucleoside phosphates locomotion, rats, spinal cord injuries, cytidylyl-3 -5 -guanosine*

Introduction

After a spinal cord injury (SCI), axons that synapsed with neurons in the lower spinal cord regenerate in animal models, but locomotion recovery did not follow regeneration. It is assumed that the animals did not learn to use their newly regrown connection.[1,2] Spinal neural networks play an important role in controlling locomotion. These spinal networks, known as central pattern generators (CPGs), are capable of producing step-like patterns in the absence of supraspinal and/or afferent inputs.[3] An effective way to activate the CPG for better learning is epidural stimulation. It has been shown that epidural stimulation enhanced hindlimb stepping in rats with complete spinal cord transections,[4] and helped to restore lower extremity voluntary control in chronic motor complete patients.[5,6] However, the mechanisms by which epidural stimulation could improve motor function are not well understood.

Learning in CPG can be performed by using a combination of epidural stimulation and training strategy on a treadmill.[7] It has been shown that widespread activation of sensory afferents, which returned to the CPG circuits, is fully dependent on the type of locomotion training on a treadmill. It is quite a possibility, because widespread activation in the spinal cord could strengthen synaptic activity and specially plasticity in lumbosacral motor centers through Hebbian mechanism.[8] Hebbian theory proposes an explanation for the adaptation of neurons in the brain during a learning process.[9] When an axon of a neural cell is placed near enough to excite another cell repeatedly, some growth process (new axonal and/or dendritic projections) or metabolic change can develop in one or both cells like increasing the efficiency of the firing cell.[9] These synaptic changes are well-known as synaptic plasticity. According to the recent studies, an afferent feedback adjusts CPG operation to provide a stable

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locomotion[10] that can play a key role in performing Hebbian learning process. The authors believe that increasing the number of involved sensory feedback can improve the quality of recovery of CPGs' functions because the Hebbian learning process gets implemented rapidly.

The claimed belief has been assessed through simulation studies on a model of CPG and experimental studies on the spinalized rats are based on two differently designed types of treadmill training in conjunction with epidural stimulation.

Materials and Methods

Simulation study

Central pattern generator model description

In this research, a model of adaptive CPG was used that was previously proposed by Righetti *et al.*[11] for simulation studies. Figure 1 shows the used model. This model was a network of adaptive coupled Hopf oscillator that was used to learn any desired periodic signal. The model was described by the following set of differential equations.[11]

$$\dot{x}_i = \gamma\left(\mu - r_i^2\right)x_i - \omega_i y_i + \epsilon F(t) + \tau \sin\left(R_i - \phi_i\right) \qquad (1)$$

$$\dot{y}_i = \gamma\left(\mu - r_i^2\right)y_i - \omega_i x_i \qquad (2)$$

$$\dot{\omega}_i = -\epsilon F(t)\frac{y_i}{r_i} \qquad (3)$$

$$\dot{\alpha}_i = \eta x_i F(t) \qquad (4)$$

$$\dot{\phi}_i = \sin\left(R_i - \operatorname{sgn}(x_i)\cos^{-1}\frac{y_i}{r_i} - \phi_i\right), \quad i \neq 0 \qquad (5)$$

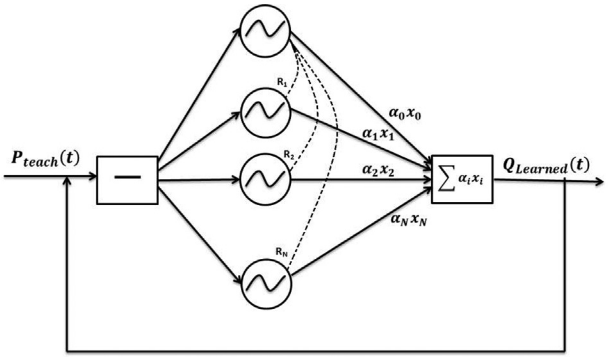

Figure 1: The structure of the network of adaptive Hopf oscillators. Each oscillator receives the same learning signal $F(t) = P_{\text{teach}}(t) - \sum_{i=0}^{N}\alpha_i x_i$, which is the difference between the signal to be learned, $P_{\text{teach}}(t)$, and the signal that already learned, Q_{learned}. Finally, to keep the correct phase differences between oscillators. All of them (except oscillator 0) receive the scaled phase input R_i from oscillator 0[11]

$$R_i = \frac{\omega_i}{\omega_0}\operatorname{sgn}(x_0)\cos^{-1}\left(-\frac{y_0}{\sqrt{x_0^2 + y_0^2}}\right) \qquad (6)$$

$$F(t) = P_{\text{teach}}(t) - \sum_{i=0}^{N}\alpha_i x_i \qquad (7)$$

where x_i, y_i were the i^{th} adaptive Hopf oscillator, and the frequency was defined by ω_i. $r_i = \sqrt{x_i^2 + y_i^2}$, η, and ϵ were positive coupling constants controlling the learning rate. P_{teach} represented the input signal to learn, and $Q_{\text{learned}} = \sum_{i=0}^{N}\alpha_i x_i$ was the learned signal that was coded in the network of the oscillator. α_i variable also learned the amplitudes of the frequency components. This system could change its own parameters to learn the frequencies of the periodic input signals. So, it could learn any range of frequencies. This adaptive mechanism could be called dynamic Hebbian learning because of its similarities with correlation-based learning observed in neural networks. For keeping the correct phase difference between the oscillators, a coupling scheme was added. All the oscillators (except oscillator 0) were capable of receiving the scaled phase input R_i, described by Eq. (6), from oscillator 0. Therefore, when the phase oscillator R_i was coupled with oscillator i, the phase-locking between oscillator 0 and i might likely to happen.[11]

Analysis of increasing the feedback weight

In this research, at first through some simulation studies on a model of CPG, the effect of increasing the feedback weight, as the afferent input weight, on the recovery of CPG was analyzed. Increasing the weight of input afferent in the CPG model could be interpreted as increasing the number of input afferents of the CPG. In this study, the CPG model described by Eqs. (1)–(7) had been used to learn the input signal (P_{teach}) describing by Eq. (8).

$$P_{\text{teach}} = 0.8\sin(15t) + \cos(30t) - 1.4\sin(45t) - 0.5\cos(60t) \qquad (8)$$

Four oscillators were used to learn the input signal, P_{teach}. The initial frequencies $\omega_i(0)$ were distributed between 6 and 70. The initial amplitudes and phase were $\alpha_i(0) = 0$ and $\varphi_i(0)$, respectively. The initial conditions were $x_i(0) = 1$, $y_i(0) = 0$, i, $\mu = 1$, $\gamma = 8$, $\eta = 0.5$, $\tau = 2$.

In Eq. (1), the ϵ was the feedback coefficient which can be considered as afferent input weight of CPG model. Increasing the ϵ would mean increasing the weight of input afferent in the CPG model, and it can be interpreted as increasing the number of input afferents of

CPG and vice versa. In this study, the amount of ϵ was changed and its effect on the learning process of the CPG model was evaluated. According to the results [Figure 2], as the amount of ϵ was 0.09, the oscillator output signal could not follow the input pattern signal P_{teach} correctly [Figure 2A]. The computed root mean square of tracking error was 0.96. In contrary, when the amount of ϵ had been increased to 0.9, the learning process became much better [Figure 2B], and the computed root mean square of tracking error was 0.47. When the amount of ϵ had been increased to 9, the network correctly had learned the input pattern [Figure 2C], and the computed root mean square of tracking error was 0.06. According to results, it can be claimed that increasing the feedback gain of CPG model can be interpreted as increasing the number of input afferent signals of CPG, and the performance of learning process became much better.

Experimental studies

In our research, all experimental procedures were performed according to the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals. Six female adult Wistar rats (200–250 g) were used for this study. The rats were anesthetized with a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg). During the procedures, a deep level of anesthesia was maintained. Supplemental doses of ketamine were administered as needed. Then a partial laminectomy was performed in all the rats at a thoracic level (T9–T11), and the spinal cord was completely transected using fine scissors and forceps. To prevent reconnection of the cut ends of the spinal cord, gel foam was inserted into the gap created by the transection.[4] Then, stimulating epidural electrodes (Silver, A-M Systems, USA) were implanted at below the L2 vertebra about 2–3 weeks before testing was initiated. The electrode wires exiting from Teflon tube that punched onto the mid-line of the L2 vertebra and then connected to a stimulator which was designed in the laboratory. A small portion (1 mm notch) of the Teflon coating of the stimulation electrodes was removed to expose the stainless steel wire on the surface facing the spinal cord. Ground wires (1 cm of the Teflon removed at the distal end) were inserted in the mid-back region subcutaneously. The electrodes were implanted about 2–3 weeks before testing was initiated. The electrode wires were punched onto the back of the rats and then connected to a stimulator which was designed in the laboratory.

Continuous epidural electrical stimulation was delivered at 40 Hz with intensity between 1^{V} and 3^{V}. Two different training protocols were designed. Six animals were assigned to two experimental groups: Three rats for

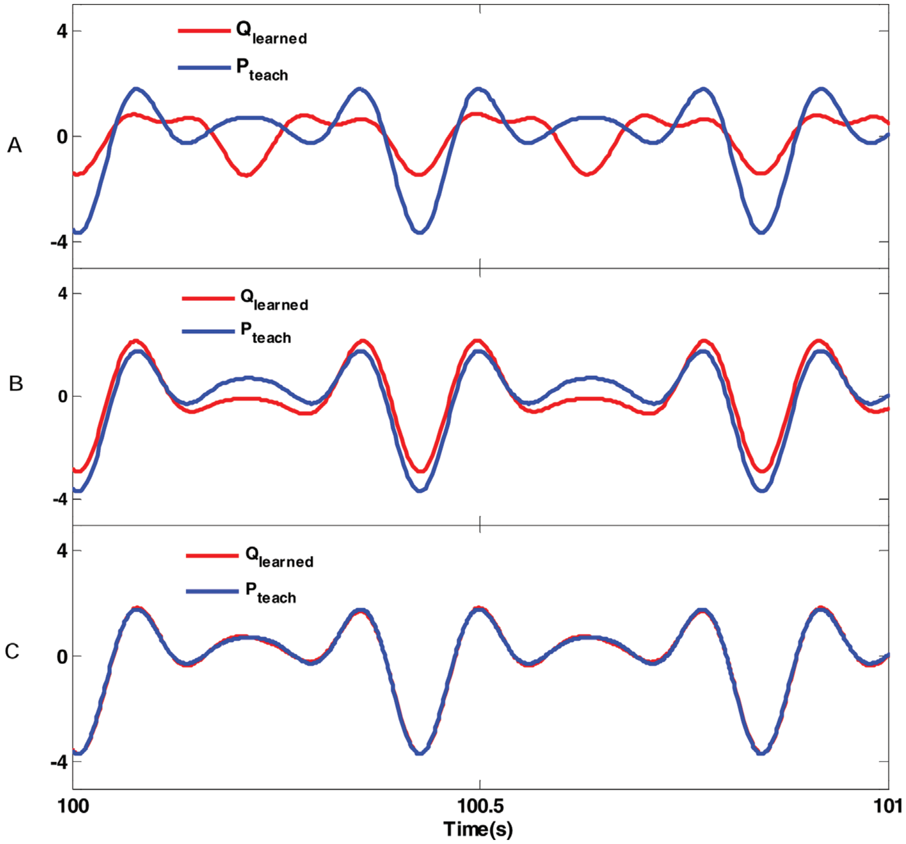

Figure 2: The reference input signal of CPG model ($P_{\text{teach}} = \mathbf{0}.8\sin(\mathbf{15}t) - \cos(\mathbf{30}t) - \mathbf{1}.4\sin(\mathbf{45}t) - \mathbf{0}.5\cos(\mathbf{60}t)$**)** and the output signal of CPG model (Q_{learned}) obtained during the learning process. (A) When the amount of ϵ is decreased to 0.09, (B) when the amount of ϵ is increased to 0.9, (C) when the amount of ϵ is decreases to 9

stimulation/biped (S/B), and three rats for stimulation/creep (S/C). For the rats in S/B group, an upper body harness support system was used to place them on a treadmill to perform bipedal locomotion and standing [Figure 3], and in the S/C group, rats could creep on a treadmill which was confined to a four walls of a cabinet with no weight support [Figure 4]. This cabinet was used to prevent the rats from falling off the treadmill. When the rats creep on the treadmill, more afferents are involved in comparison to the situation, in which the rats perform bipedal locomotion. Each group was trained for about 20 min, 5 days per weeks in 1 month, and the epidural stimulation used for both groups during treadmill training with the speed of 11 cm/s.

Experimental result

Three qualitative scales were envisioned to assess the improvements in overground stepping in their cage [Table 1].[12] The result of training in both groups shows that the group S/C had considerably better overground movement after 3–5 sessions even though the group S/B had much better hindlimb locomotor activity during the training process. In other words, the rats in group S/C had much better improvements in overground stepping in their cage.

Figure 3: Bipedal locomotion while an upper body harness support system was used to place the rat on a treadmill.

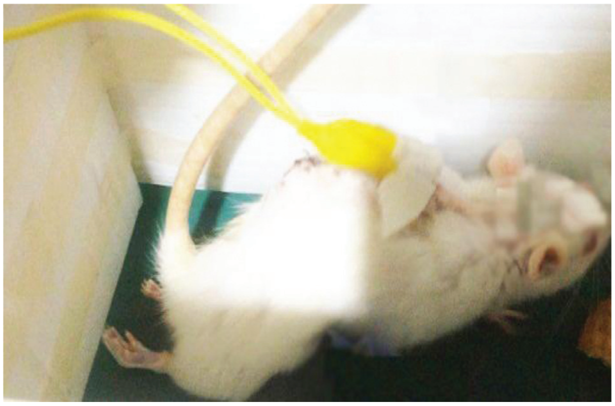

Figure 4: Creeping on a treadmill while it is confined to four walls of a cabinet with no weight support.

After 1 month of training, qualitative scales were computed [Table 2]. The assigned qualitative scales to the three rats in S/B group were 2, 2, and 2, respectively. Also, the assigned qualitative scales to the three rats in S/C group were 1, 1, and 0, respectively. Consequently, the qualitative assessment showed that the rats in group S/C had more reliable and stable movement in comparison to the group S/B. Therefore, training protocol involving more afferents could expedite the movement recovery. These results coincided with what were concluded through simulation studies.

Discussion and Conclusion

In this study, it was shown that with increasing the number of involved sensory afferents, we could accelerate the recovery of CPGs' function during training in conjunction with epidural electrical stimulation. We believe that in this situation, it can be expected that the Hebbian learning process could be implemented more rapidly. At first, some simulation studies on a model of CPG elucidated that increasing the gain of input feedback increases the learning accuracy of CPG model. Increasing the gain of input feedback can be interpreted as increasing the number of input afferents. In the next step, this idea was assessed through some experimental studies.

In the recent studies,[4] step training with weight support is a usual form of activity-based rehabilitation for SCI rats.

Table 1: The qualitative scales envisioned to assess the improvements in overground stepping of rats in their cage

Qualitative scale	Description
0	Rat cannot use the hindlimbs and they are dragged on the floor during the stepping
1	Rat can sometimes use the hindlimbs weakly during the stepping
2	Rat can continuously use the hindlimbs stable during the stepping

Table 2: The qualitative scales assigned to the rats of each experimental group after 1 month of training. Three rats were assigned to each group

Rat number	S/B group	S/C group
1	1	2
2	1	2
3	0	2

Similar to clinical studies, only modest, task-specific improvements in treadmill stepping occurred during step-training animal models of incomplete SCI which was rarely led to improvements in overground stepping. In this study, the effectiveness of a new training protocol along with epidural electrical stimulation on movement recovery of SCI rats had been evaluated. The new training protocol was designed in a way that more sensory afferents were involved. According to the proposed protocol, the rats have been creeping on a treadmill. After 1-month training, the maximum evaluation scale assigned to the rats trained according to the conventional protocol, proposed in previous research,[4] was 1, and the evaluation scale assigned to all rats trained according to the proposed protocol was 2. In other words, it was shown that treadmill training without any weight support which rats can creep freely on a treadmill has much better improvement in overground movement than the ones with weight support. Therefore, locomotion recovery can expedite in spinalized rats when instead of bipedal training with weight support, the rats creep on a treadmill similar to the locomotion of healthy ones. Since during the free creeping, more afferent nerve fibers fire the CPG circuits; such experimental results support the achieved simulation results. Therefore, it can be concluded that locomotion recovery can expedite in spinalized rats when instead of bipedal training with weight support, the rats creep on a treadmill because more sensory afferents are involved during such training. The achieved results can be interpreted based on the Hebbian learning rule, because if more sensory afferents are involved during training, the Hebbian learning process can be implemented more rapidly.

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Conflicts of interest

Authors' contributions: HRK carried out the design and coordinated the study, conducted the experiments on the animals and prepared the manuscript. ZK provided assistances in design of the study and contribute to the result analysis and preparation of the manuscript. AM, as the neurophysiologist and advisor of the study, provided assistance in the design of the experiments and performance of surgical procedures.

There are no conflicts of interest.

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