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The System for Measuring Gait Parameters and Rehabilitation of Spinal Rats

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Spinal cord injury results in serious neurophysiological consequences that alter healthy body functions and devastate the quality of life. One of them is the loss of sensorimotor functions, including disturbance or complete loss of locomotion. Recovery of locomotor function is one of the primary goals of therapeutic interventions in the case of an animal model. Choosing the right treatment strategy is crucial to the rehabilitation process, which is usually assessed by using quantitative methods. That is why it is important to have the technical means to quantify pertinent locomotion changes in experimental animals. The most useful quantitative methods can be divided into a few groups, such as electromyography, kinematics, and kinetics methods. Yet, despite the fact that each of the methods gives reliable data, it seems that only the combination of results taken from different methods gives a holistic view of the realistic level of restoring the locomotion. However, it is difficult to apply all the mentioned methods at the same time during each single examination. The presented article describes the application of the specialized system to the measurement of important locomotion parameters taken from different methods for the cases of spinal cord injury rats. Data were collected during each examination run. The study was carried out on spinal cord injury rats with controlled injury to the spinal cords of the lumbar section. Due to paraplegia, also a body weight support system was applied. Locomotion on the treadmill was induced by stimulation of the central pattern generator.

topics: rehabilitation, SCI rats, gait analyses, locomotion restore

1. Introduction

Traumatic *spinal cord injury* (SCI) is an event that, in the majority of cases, results in the loss of sensorimotor functions, as well as autonomic deficits [1]. The primary injury is the result of damage to cell membranes and disruption of the blood–brain barrier, and its consequence is vast neurodegeneration in the area of the damage and its vicinity [2, 3]. The secondary injury manifests in various movement disorders, including disturbances or complete loss of locomotion. If the higher spinal segment is injured, then the greater area of the body is affected by sensorimotor disorders [4]. Once the injury area has been properly secured and the neurodegenerative processes have been stopped, the appropriate rehabilitation process can begin, which is closely related to a dedicated rehabilitation strategy, and its main goal is to restore lost sensorimotor functions [5].

Animal models play a key role in understanding the functioning of the central nervous system and are very helpful in finding and determining the appropriate rehabilitation strategy after spinal cord injuries [6]. However, the selection of the rehabilitation strategy involves many problems, including technical ones. The most important problem is the absence of information transfer through the ascending and descending pathways. That is why locomotion is impossible without appropriate support and stimulation [7]. Due to hind limb paresis, it is necessary to relieve the limbs by applying a *body weight support* (BWS) system to provide proper distance between the body and the ground. These kinds of systems have been used previously in studies on SCI rats and consist of a chest harness in which the animal is dressed, a supporting arm, and ropes connecting the animal with the arm [8]. For an animal prepared in this way, it is possible to induce locomotion by stimulating the *central pattern generators* (CPG). CPGs are the neuronal structures located in the lumbar regions of the vertebrate spinal cord, and their characteristic feature is the ability to produce rhythmic motor patterns such as walking [9]. These neuronal sub-networks can be activated not only from the brain structures by descending inputs but also from certain *peripheral nervous system* (PNS) areas by touch, pressure, or rubbing [10]. The second method that can activate the CPGs is direct electrical stimulation of the proper lumbar spine section [11].

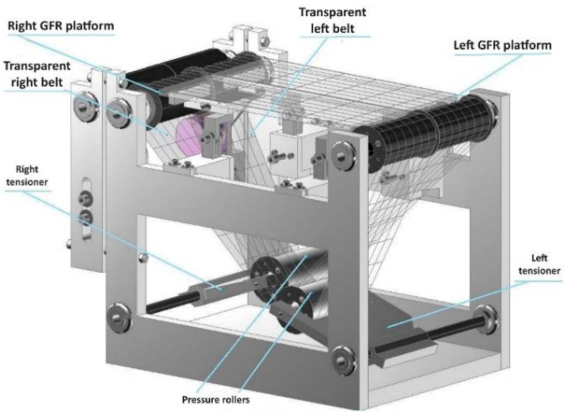

Fig. 1. The transparent treadmill for SCI rat training.

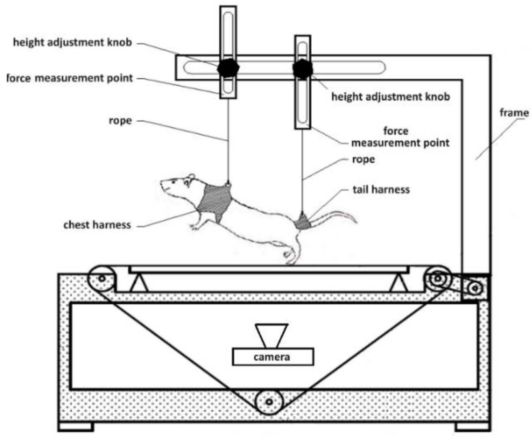

Fig. 2. The BWS system for SCI rat training.

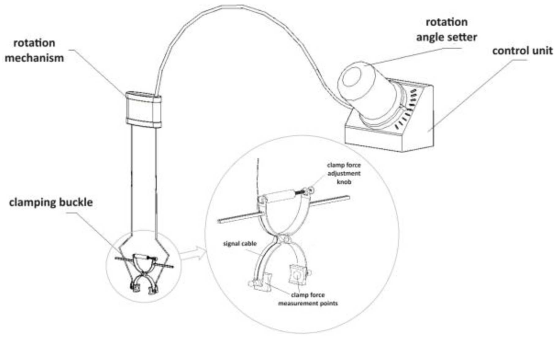

Fig. 3. The mechanical stimulator to induce the SCI rats walking effect.

Measurement gait parameters methods can be divided into two basic groups: qualitative and quantitative methods. Qualitative methods focus on observation or video recording of the measurement run and then subjective assessment of the current gait quality. The results usually describe whether the gait is better or worse compared to the last exercise or to the gait quality before the injury. These techniques include, e.g., the Tarlov method and the Basso, Beattie, and Bresnahan (BBB) method; however, because of their low sensitivity, they are limited to usage in mild SCI injury cases, in which it is not necessary to monitor subtle movement changes [12, 13]. Quantitative methods are applied using more or less sophisticated technical tools, and the resulting data is typically precise and unambiguous. One of the major tools to assess gait is *electromyography* (EMG), involving the implantation of electrodes into specific muscles and receiving EMG signals generated during walking [14]. The main disadvantage of this method is its invasiveness, but the main advantage is its ability to record the activity of flexors and extensors during locomotion and determine muscle synergy. The fundamental parameters acquired by this technique include the temporal relationships between individual muscle reactions, signal durations, amplitudes, or burst shapes. Another technical limitation is a wired connection between the animal's body and the amplifier, which is often troublesome during performing gait tests. The second group of methods used to parameterize SCI rats' gait is a kinematic analysis, which allows to obtain gait parameters such as gait's speed, number of steps, angle changes in the examined joints, acceleration of the body segments, plantar position of the foot relative to the ground, the way of placing the foot on the ground, and the others. The third group of measurement methods are kinetic methods, which allow for the determination or direct measurement of forces and moments of force during locomotion. One of the basic parameters here is the *ground reaction force* (GFR) as the main indicator of locomotion abilities. The limitation is that the animal must pass through a measuring platform of limited length, which allows for the measurement of only a few steps.

Each of the aforementioned methods produces data for parameterizing the SCI rat's gait; however, all of them, despite their reliability, have certain limitations. The EMG method, due to the cable connection between the rat and the measuring amplifier, requires the use of a treadmill or moving the amplifier system parallel to the walking animal, which can be inconvenient or even dangerous. Therefore, the simultaneous use of the EMG method and the kinetic method (GFR platform) is difficult to implement. Moreover, kinematic methods used on SCI rats do not fully determine the actual locomotion capabilities, because the animals are suspended by the BWS system, so their hindlimbs are relieved. Therefore, the gait may seem quite smooth, and the kinematic data may indicate that the gait is close to a stereotype, which may falsely indicate recovery of locomotion functions. For that reason, the relieving force should also be known. In connection with the above, it seems that only the use of all methods simultaneously during each test would show a comprehensive view of

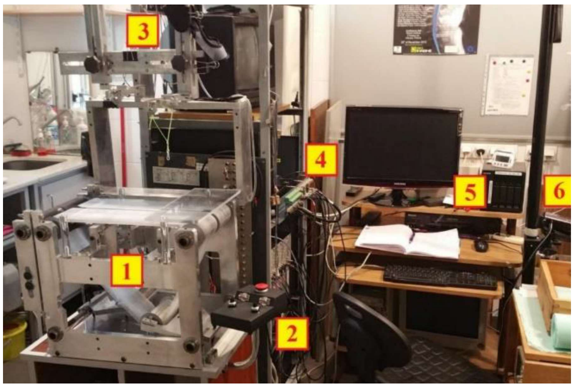

Fig. 4. The research and training station for spinal rats (front view): 1 — treadmill with built-in camera, 2 — control panel with emergency button, 3 — BWS system, 4 — multi-channel amplifier, 5 — computer, 6 — camera on a tripod.

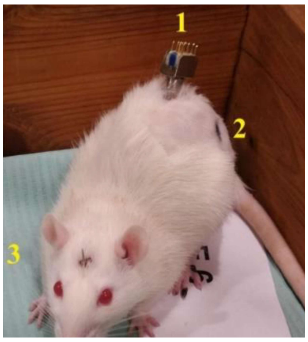

Fig. 5. The rat prepared for the test: 1 — EMG output port, 2 — marker placed on hip joint, 3 — cannula for drugs.

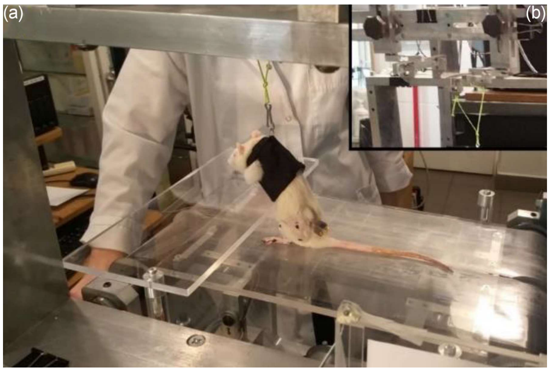

Fig. 6. Adapting the rat to the research station; (a) the rat is standing on the treadmill and connecting to the BWS, (b) knobs of the BWS system adjustment.

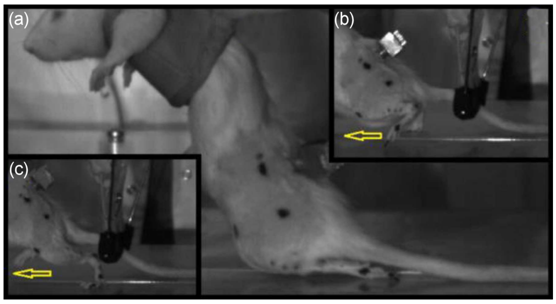

Fig. 7. An example of gait inducing by using a clamping buckle. (a) Without the tail buckle, hind limbs are rubbing against the treadmill's surface. No walking effect. (b) Tail squeezing by buckle. The left hind limb swing phase is visible. (c) Tail squeezing by buckle. The right hind limb swing phase and the left hind limb stance phase are visible.

locomotion capabilities and make it possible to observe even subtle changes in movements and locomotion. Attempts are being made to build advanced measurement stations in which the inconveniences described above could be eliminated [15, 16]. These modern laboratory stations are usually technically advanced systems with sophisticated technical solutions. The presented article describes the structure, capabilities, and application of the specialized system to the measurement of important locomotion parameters taken from different methods for the cases of SCI rats.

2. Materials and methods

2.1. Main system components

The manufactured system consists of the measuring treadmill, the BWS system, a mechanical stimulator, an EMG amplifier, an electronic control system, a camera, a fast camera, and dedicated software. The treadmill (Fig. 1) has been made of transparent materials so that the rat's gait could be recorded from underneath. The measuring platforms are independent for the right and left limbs.

The manufactured body weight support system allows the rat to be suspended both during bipedal and quadrupedal locomotion, and its scheme is shown in Fig. 2. Quadrupedal locomotion requires clothing the rat with two harnesses, i.e., the chest one and the tail one. When the rat is moving on its hind limbs, only a chest harness is required. The knobs are used to set the correct body position in relation to the treadmill surface, which enables making gait tests in the entire range of body positions, from a horizontal to an upright posture. The animal is suspended by ropes connected to force meters, so the value of forces necessary to relieve the animal is known.

In order to induce CPG activation, a self-designed mechanical stimulator has been made (Fig. 3). SCI rats's CPG activation can be induced by physical tail stimulation in the perianal area. Typically,

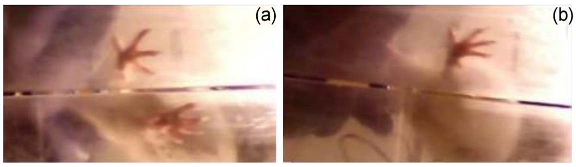

Fig. 8. An example of a bottom gait recording: (a) double support hind limbs phase, (b) swing phase of left hind limb, stance phase of right hind limb.

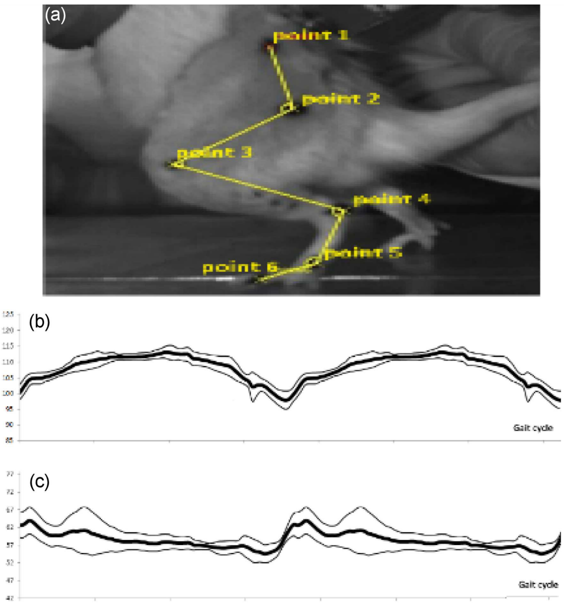

Fig. 9. An example of kinematic analysis using the TEMA software: (a) determining the marker coordinates and angle changes in selected joints; points 1, 2, 3 — hip joint, points 2, 3, 4 — knee joint, points 3, 4, 5 — ankle joint, points 4, 5, 6 — metatarsal; (b, c) the averaged angle changes in selected joints of the hind limb of the SCI rat, respectively for hip joint and knee joint.

activation occurs as a result of two-point tail pinching (e.g., by using the thumb and index finger) [17]. The clamping buckle ensures proper tail compression, and the built-in strain gauges allow adjusting the proper clamping force.

The designed and manufactured research and measurement station is shown in Fig. 4. All previously described measurement methods can be used simultaneously during one SCI rat's test. A multichannel amplifier and software are responsible for processing the EMG signal. The kinetic method is implemented to measure the GFR and to measure the force needed to relieve an animal. The implementation of the kinematic method involves recording rats's gait both from under the surface of the treadmill and with the high-speed camera (100 fps). The collected data is then processed and integrated using dedicated software.

2.2. Rat preparation and the rehabilitation strategy

All surgical and experimental procedures were performed at the Nencki Institute of Experimental Biology PAS and were conducted with the approval of the First Local Ethics Committee in Poland, according to the principles of experimental conditions and laboratory animal care of the European

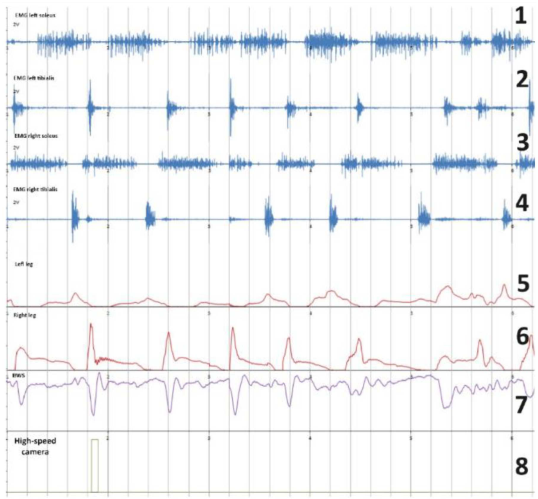

Fig. 10. An example of raw data summary: 1 — EMG activity of left soleus extensor, 2 — EMG activity of left tibialis flexor, 3 — EMG activity of right soleus extensor, 4 — EMG activity of right tibialis flexor, 5 — GRF for left hind limb, 6 — GRF for right hind limb, 7 — BWS holding force, 8 — high-speed camera pulse synchronizer.

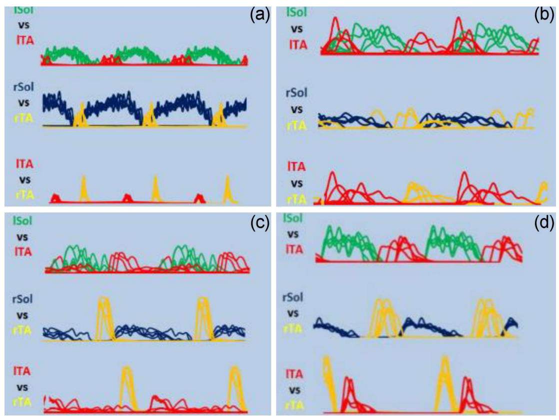

Fig. 11. EMG patterns in the soleus extensor (Sol) and tibialis anterior flexor (TA) muscles for the left (l) and right (r) limbs. Rectified and filtered EMG records, normalized to the step cycle, show the left-right and flexor-extensor coordination over the same step cycles: (a) healthy rat; (b–d) SCI rat during the first, second, and third sessions, respectively..

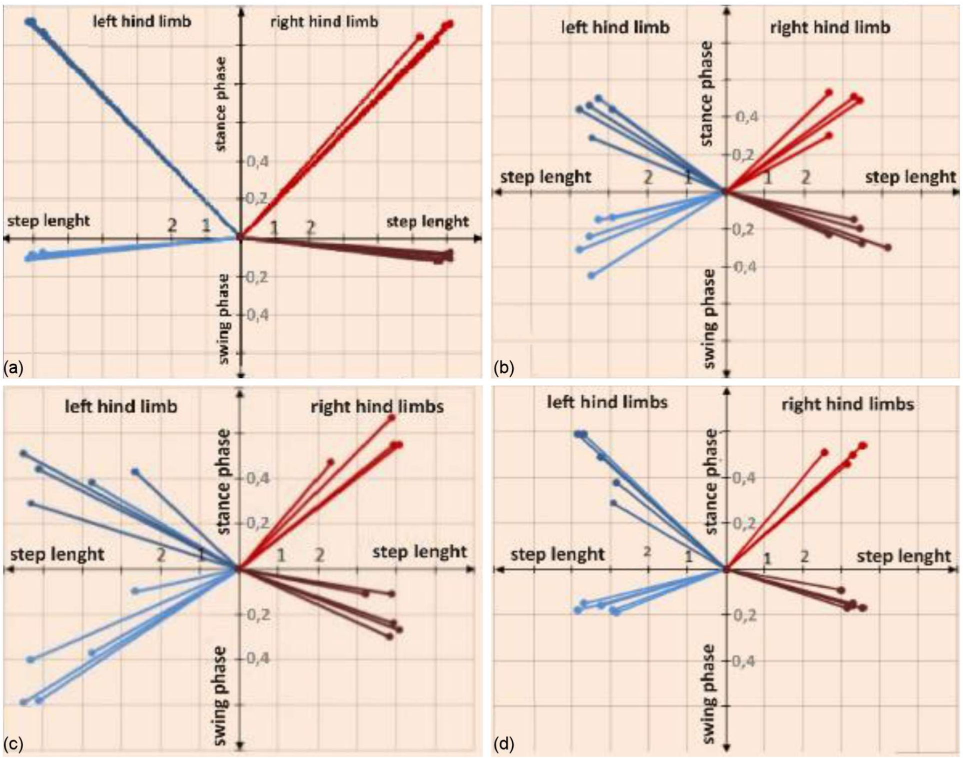

Fig. 12. The duration of individual phases of the gait cycle as a function of step length: (a) healthy rat; (b–d) SCI rat during the first, second, and third sessions, respectively.

Union and the Polish Law on Animal Protection. The study involved Wistar Albino Glaxo (WAG) rats with a completely transected spinal cord at the thoracic level Th9/10. In the primary injury period, bipolar EMG recording electrodes were implanted in the soleus and tibialis anterior of both hind limbs, and the communication port was placed on the animals back. The secondary injury period included research aimed at restoring lost sensorimotor functions, including locomotion exercises. The rehabilitation strategy, in addition to treadmill exercises, also included determining the effect of serotonergic agonists administered during exercise on the hindlimb locomotor abilities. An example of rat prepared to begin a series of tests is shown in Fig. 5. The same rat adaptation to the measuring equipment is shown in Fig. 6.

2.3. Experimental research and test series

The system was tested for training and measuring gait parameters of three SCI rats in three training sessions. Each of the sessions involved suspending the rat to the BWS system and inducing the best possible walking effect on the hind limbs. The force needed to induce the best possible gait was different for each rat but ranged around 1.2 N. In each subsequent session, the same force value was set so that the stimulus had the same value in each of the three sessions. Examples of the recording's frames from the high-speed camera showing rat locomotion with and without stimulation are presented in Fig. 7.

The training lasted from 5 to 10 min, during which kinetic parameters (GRFs, BWS holding force) were measured and EMG were recorded. At the same time, the gait was recorded under the treadmill surface (Fig. 8) and by the high-speed camera for kinematic analysis reasons (Fig. 9).

After training, the data was processed using software specially developed for this purpose. All data timelines were synchronized. An example of a summary of raw data is shown in Fig. 10.

2.4. Raw data processing and results presentation

Based on the raw data collected during training sessions, ten parameters that clearly describe gait were determined. Examples of selected three of them are provided below. All presented examples were determined at the treadmill belt speed of 5 cm/s for a sample of five consecutive gait cycles. The first parameter shows how the muscles are controlled. This form of presenting results is simple and quick in assessing an important parameter — muscle synergy (Fig. 11).

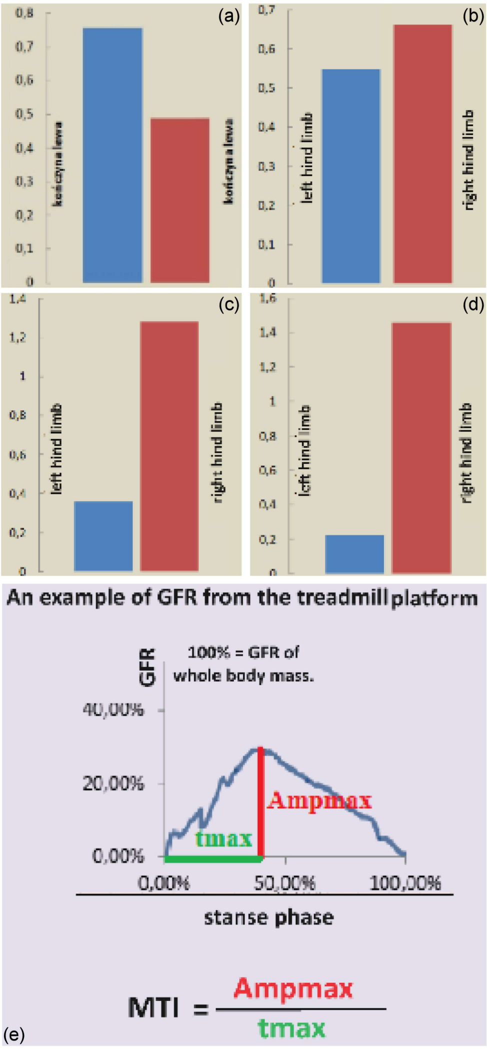

Fig. 13. The mass transfer index (MTI); (a) healthy rat; (b–d) SCI rat during the first, second, and third sessions, respectively; (e) MTI definition.

The second parameter is described as the duration of individual phases of the gait cycle as a function of step length (Fig. 12). This form of results presentation allows for a quick and accurate assessment of, e.g., gait symmetry.

The third parameter is the mass transfer index (MTI) and is shown in Fig. 13. This parameter indicates how the body weight is transferred.

3. Conclusions

There appear to be no simple methods to assess the recovery of locomotion and overall rehabilitation progress after spinal cord injury in rats. Each of the methods mentioned in the article focuses on a given aspect of locomotion (generated forces, muscle activity, gait kinematics) and in this sense each of them is effective. However, a comprehensive view of the rehabilitation progress requires the use of all methods to accurately determine even the smallest changes in the locomotion. Therefore, attempts are being made to create specialized research stations that can collect as much data as possible from various measurement methods. The manufactured research station and the proposed way of result presentation make the system a convenient tool for training and gait parameter measurements of SCI rats. First, the method of activating central pattern generators and inducing locomotion was automated by using the buckle clamp. Moreover, it is a non-invasive method of stimulating CPGs, and, unlike electrostimulation, it does not require stimulating electrode implantation surgery. Secondly, one study uses kinetic, EMG, and kinematic methods, which certainly saves time and allows the comparison of different types of data. The amount of measurement data obtained during training means that other cases of locomotion disorders can also be tested using the system, e.g., in the cases of a non-complete spinal cord injury, when BWS is often not required. The combination of results from various methods in the form of parameters and indexes allows for easy determination of rehabilitation progress after spinal cord injury in rats. Although the specificity of the research included hind limb locomotion, the BWS system is also adapted to quadrupedal locomotion. Therefore, the system is a universal solution.

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