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Research report

Recovery of overground locomotion following partial spinal lesions of different extent in the rat

Teresa Górska[a,*], Barbara Chojnicka-Gittins[a], Henryk Majczyński[a], Wojciech Zmysłowski[b]

[a] Department of Neurophysiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteura Street, 02-093 Warsaw, Poland
[b] Institute of Biocybernetics and Biomedical Engineering, Polish Academy of Sciences, 4 Ks. Trojdena Street, 02-109 Warsaw, Poland

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ABSTRACT

In six rats with incomplete low thoracic spinal cord lesions of different extent, basic gait indices such as locomotor speed, step cycle duration, soleus (Sol) muscle activity duration, the interval between the onsets of Sol and tibialis anterior (TA) muscle activities and interlimb coordination were investigated by EMG analysis of the Sol and TA muscles recorded using chronic electrodes. The operated animals were divided into two subgroups: (1) those with a smaller lesion involving the dorsal quadrants of the spinal cord and, to a variable extent, the ventrolateral funiculi, and (2) those with an extensive lesion sparing only parts of the ventral funiculi. The locomotion of all rats was tested once a week for the first 5 weeks postsurgery and then once or twice a month, up to 3.5 months. The surgical lesions affected all analyzed gait indices: the locomotor speed decreased, while all other indices increased compared to recordings made preoperatively. In both subgroups the major improvement in locomotion occurred within the first 5 weeks following surgery and the rats reached a plateau in their recovery at around 2 months postoperatively. The late effects of injury depended on the severity of the spinal lesion: in the subgroup of rats with a smaller lesion, the postoperative changes in the different indices amounted to approximately 20%, while in the subgroup with extensive lesions this was increased by 20–50%, with changes in various indices being strongly correlated with the extent of the injury in individual animals. These postoperative changes were partly due to alterations in the relationships between the analyzed variables.

1. Introduction

In the last decade, rodents and especially rats have been the preferred animal model in studies on the effects of spinal cord injury (SCI) on locomotion. In adult rats, complete transection of the spinal cord at the low thoracic level (T_8–T_{10}) abolishes hindlimb locomotor movements [5,6,25,35]. These spinal animals move around using their forelimbs, while the hindquarters and the hindlimbs are dragged behind along the ground. The EMG activity of two hindlimb muscles, the soleus (Sol) and the tibialis anterior (TA), recorded during locomotor-like movements of the hindlimbs induced by tail pinching in spinal rats suspended over a treadmill, indicated substantial abnormalities in burst duration, overlapping of flexor and extensor muscle activity and coupling of the activities of the muscles in the right and left hindlimbs [25,35].

Following incomplete lesions of the spinal cord at the low thoracic level, rats can regain spontaneous quadrupedal walking after a period of recovery. In general, comparisons of hindlimb motor abilities after various partial spinal lesions have shown that the ventral and ventrolateral parts of the spinal white matter are crucial for locomotion, while the dorsal part plays a less important role [23,24,34]. Sparing a small amount (approximately 10%) of fibres in the ventral and ventrolateral funiculi permitted the spontaneous return of weight-supported stepping and thus allowed quadrupedal walking, although with obvious deficits [2,20,34,40]. In contrast, sparing a much greater proportion of the white matter in the dorsal part of the spinal cord, with total destruction of the more ventral parts, did not lead to recovery of hindlimb body weight support [34]. Lesions confined to the dorsal half of the spinal cord resulted in transient motor impairment, usually lasting up to 4 weeks [15–17], while following destruction of the corticospinal tract at the medullary level, hindlimb movement deficits usually recovered within the first week after surgery [27,30].

Comparison of locomotor deficits elicited by various partial spinal lesions and the rate and extent of their spontaneous recovery is, however, often hampered by differences in the lesions, postoperative survival times and methods used to assess hindlimb impairment. Several tests and methods have been designed to describe deficits in rat locomotion based on the assessment of locomotor behaviour in different experimental situations

* Corresponding author. Tel.: +48 22 659 85 71; fax: +48 22 822 53 42.
E-mail address: t.gorska@nencki.gov.pl (T. Górska).

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[9,21,28,29,33], of which the most frequently applied in recent years is the 21-point Basso–Beattie–Bresnahan (BBB) open-field locomotion scale [1]. Some more sensitive tests such as kinematic analysis [3,8,27,28], electromyographic recording of muscle activity [3,19], or the analysis of ground reaction forces during locomotion [30,31,38,39] have been used in a limited number of studies. The same applies to the analysis of gait indices such as the step cycle, stance and swing phase duration, which have not, to our knowledge, been analyzed in detail in overground locomotion, (see, however, ref. [14]), although recently some of these indices have been used to study the effects of incomplete spinal injury [16,17,20]. In addition, locomotion on a treadmill has been investigated in some studies, while in others, overground locomotion was examined, either in the open-field, [1] or on various types of runway [21,22,26,33].

The present investigation was designed to study the spontaneous long-term functional recovery of hindlimb movements during unrestrained overground locomotion in adult rats with low thoracic partial spinal lesions sparing only parts of the lateral and/or ventral funiculi. The method of recording the EMG activity of soleus and tibialis anterior muscles as rats moved on a flat runway was applied. In previous studies of rats with lesions of similar severity, their recovery was investigated mainly by behavioural testing with the BBB locomotor scale [1] for a period of 4 weeks [2,23,24,34], and rarely longer [20,40]. Our results show that the major recovery of hindlimb locomotor movements occurred within the first 5 weeks postsurgery, and a plateau of recovery was reached after approximately 2 months. The main long-lasting effects on motor performance were reduced speed of locomotion, increased duration of EMG indices related to the stance phase – greater than would be expected from changes in the step cycle duration due to the slowing of locomotion (see also ref. [14]) – and some deficiencies in interlimb coordination. These alterations were correlated with the extent of damage to the lateral and ventral funiculi.

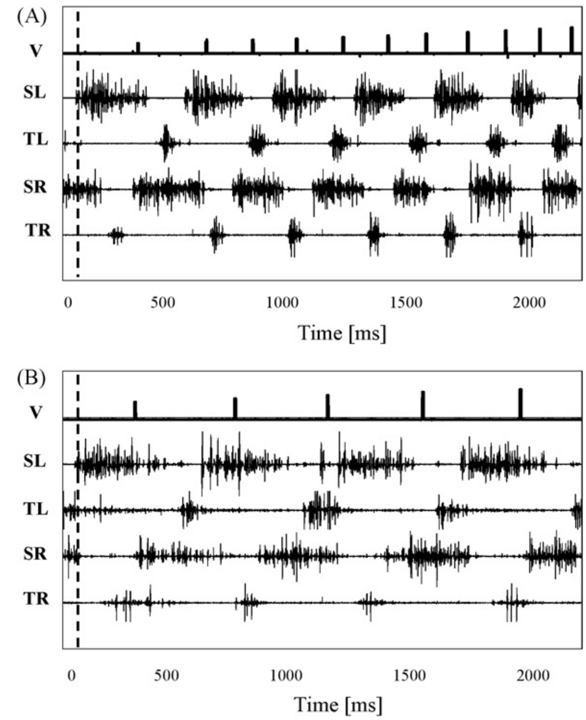

Fig. 1. Examples of EMG activity recordings for rat R6 during a sequence of steps performed along a runway before surgery (A) and 17 days after an extensive lesion of the spinal cord (B). SL, SR and TL, TR denote the left and right soleus muscle and the left and right tibialis anterior muscle, respectively. V, signals from photocells placed every 10 cm along the runway. The vertical dotted line denotes the reference time which was the onset of the left soleus (SL) muscle activity.

2. Materials and methods

2.1. Subjects

The experiments were performed on six adult male Wistar rats, aged 3 months at the start and weighing between 305 and 350 g. The animals were kept in separate cages in a room with a 12:12 h dark/light cycle and received water and food *ad libitum*. Rats were subjected to incomplete spinal cord injury and implantation of chronic electrodes into the soleus and tibialis anterior muscles of both hindlimbs to record EMG signals during overground runway locomotion, before and after injury. The locomotion of some of these rats, tested 3 months postsurgery using contact electrode recording, has been described previously [14]. All experiments were conducted with the approval of the Local Ethics Committee at the Nencki Institute and followed EU guidelines on animal care.

2.2. Implantation of EMG recording electrodes

Bipolar EMG recording electrodes were implanted into the Sol and TA muscles of both hindlimbs under Equithesin (0.4 ml/100 g b.w. administered i.p.) anaesthesia. The electrodes were made of Teflon-coated stainless steel wire (0.24 mm in diameter; AS633, Cooner Wire, Chastworth, CA, USA). The tips of the electrodes with 1–1.5 mm of the insulation removed were pulled through a cutaneous incision on the back of the animal, and each of the hook electrodes was inserted into the appropriate muscle and secured by a suture [36,37]. The distance between the electrode tips was 1–2 mm. The ground electrode was placed under the skin on the back of the animal, some distance from the hindlimb muscles. The connector with the other ends of the wires fixed to it, covered with dental cement (Spofa Dental) and silicone (3140 RTV, Dow Corning), was secured to the back of the animal. A wire loop left under the skin on the back of the animal prevented the electrodes from being pulled from the muscles during movement. After surgery, the animals were given antibiotics to reduce the risk of infection. EMG recording started about 1 week after electrode implantation. The position of the electrodes in the muscles was verified visually after the animals were sacrificed.

2.3. Experimental procedure

Before surgery the animals were taught to move freely along a runway 2.5 m long and 12 cm wide, situated 1.5 m above the floor, using a shelter-seeking paradigm (for details see refs. [12–14]). EMG signals from the Sol and TA muscles after amplification (band pass 0.1–1 kHz) were converted a/d with a sampling frequency of 2 kHz. The step cycle duration and the duration of muscle activities were determined from the corresponding onset and offset of signals. The locomotor speed was measured using photocells mounted every 10 cm along the runway. The time and speed errors did not exceed ±2 ms and 0.25 cm/s, respectively.

The experiments with EMG recording in intact animals were preceded by a 2-week period during which the animals were familiarized with the experimental situation and taught to move along the runway at a relatively constant speed.

For each animal, one experimental session consisted of 15–20 passes along the runway. From each pass a sequence of 8–10 regular steps was analyzed, which gave a sample of at least 100 steps collected during each session for every rat. For the intact animals, data for the last session prior to the surgery was taken for detailed analysis.

Postoperatively, the quadrupedal walking ability and hindlimb movement deficits during spontaneous locomotion on a wooden surface were visually monitored every other day for each rat, until they regained hindlimb body weight support and plantar stepping, and thereafter, every 2 weeks or once a month on the runway. The experiments with EMG recording were resumed 3–7 days after the animals became able to support their body weight on the hindlimbs and were performed once a week during the first 5 weeks postsurgery, and thereafter, once or twice a month up to 3 or 3.5 months postoperatively. An example of EMG signals recorded in a rat prior to and after an extensive spinal cord lesion is shown in Fig. 1.

2.4. Data analysis

The aim of the data analysis was to characterize the animals' hindlimb locomotor movements and the activity of the Sol and TA muscles, and their coordination. A number of indices were analyzed: (1) the locomotor speed (V); (2) the hindlimb step cycle duration (T_C: the time elapsed between the onsets of two consecutive Sol muscle EMG bursts); (3) the duration of Sol muscle activity (T_{SOL}: the time between the onset and the offset of the EMG burst, and its relative duration i.e. the duty factor – DF_{SOL}); (4) the time interval between the onsets of Sol and TA muscle activities (T_{S-TA}) and its duty factor (DF_{S-TA}); and (5) the relationships between these indices. Interlimb coordination was assessed by the homologous shift and the correlation between indices in individual limbs. The duty factors were included in the analysis because they characterize the relative percentage of time taken by Sol muscle activity and by the S–TA interval in the step cycle, and thus permit comparison of the step cycle structure and postoperative changes in animals moving at different speeds. In