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Research report

Changes in forelimb–hindlimb coordination after partial spinal lesions of different extent in the rat

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HIGHLIGHTS

- Effects of spinal lesions on forelimb–hindlimb coordination were studied in adult rats.
- Footprints were recorded up to 3.5 months during locomotion along an extended walkway.
- Rats with 26–73% of SWM regained locomotor speed, stride length and gait regularity.
- Coordination was shifted in lesion dependent manner from trot to walk with Aa pattern.
- The extent of recovery suggests injury induced reorganization in the spinal cord circuits.

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ABSTRACT

Forelimb–hindlimb coordination in adult rats moving freely along 2 m long runway was investigated using the method of footprint recording. Rats were divided into 3 groups with different extent of spinal lesions (T_9). Before surgery rats moved with a mean locomotor speed of 73 ± 20 to $96 \pm 18\,cm\,s^{-1}$, stride lengths of 17.5 ± 2.0 to 21.2 ± 2.0 cm, and trot like coordination. Early after surgery the locomotor speed and the stride lengths were decreased. The forelimb steps were shorter than hindlimb steps, which led to the occurrence of unpaired forelimb steps. Unpaired steps occurred when the hind paw print lay more than half the hindlimb stride length in front of the ipsilateral paw. The number of unpaired steps was negatively correlated with the difference between the fore- and hindlimb step lengths. The recovery of locomotor speed, stride length, and step sequence patterns took up to 3.5 months depending on the extent of lesion. In the last testings the coordination was characterized by increased distances between ipsilateral footprints leading to a change from an almost synchronized trot to a lesion-dependent walk. This change was accompanied by a switch from the use of both patterns A and C to the most frequent use of the Aa pattern that is better adapted to maintain the body balance. All locomotor changes depended on the extent of the injury of lateral and ventral funiculi. These results demonstrate that footprint analysis can be used for the evaluation of forelimb–hindlimb coordination after spinal lesion in rats.

1. Introduction

In the last decade, rodents and especially rats have been the preferred animal model in studies on the effect of spinal cord injury (SCI) on locomotion. Several tests and methods have been designed to describe deficits in rat locomotion based on the assessment of locomotor behavior in different experimental situations [1–4], using different locomotor indices [5–10] and various recording methods [11–17].

One feature of particular interest in quadrupedal locomotion is forelimb–hindlimb coordination. Normal animals exhibit a one-to-one relationship between the movements of the fore- and hindlimbs, and the step cycle durations and/or stride lengths in these limbs are equal. After spinal injury, the forelimb–hindlimb coordination is impaired. This phenomenon has been mainly studied in cats [18–27]. In this species, three lesion-dependent types of coordination impairment have been differentiated during unrestrained locomotion: (1) a change in locomotion toward pacing with the preservation of equal rhythms in both girdles, (2) episodes of fore- and hindlimb rhythm dissociation, and (3) permanent dissociation of rhythms [26,27].

Forelimb–hindlimb coordination during locomotion has been less frequently studied in rats, and different tests and indices have been used to quantify the impairment and recovery of coordination (cf. [8]). The most commonly used test is the 21-point Basso–Beattie–Bresnahan (BBB) open field locomotion scale

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[28,29]. The observed reduction in BBB scores to 12–14 in rats tested after SCI indicates that such injuries affect forelimb–hindlimb coordination [7,9,10,30,31]. However, the results obtained using this procedure have often been inconsistent and controversial. For example, in one study, bilateral dorsal hemisections were found to disrupt forelimb–hindlimb coordination with no signs of recovery for several months [9], whereas in another they produced only transient impairment [7]. Similar discrepancies were found after moderate contusion injury [30,31]. In the study of Kloos et al. [10] the threshold for consistent coordination was associated with the sparing of more than 25% of the white matter. A potential limitation of BBB testing is its subjective scoring system, as shown by comparison of BBB scores recorded in the open field and their objective assessment in CatWalk testing [8,32].

Another method of assessing forelimb–hindlimb coordination was proposed based on the concept of a regular step pattern, i.e. the order of limb recruitment for forward locomotion in normal rats [33]. Six regular step patterns have been identified during walking in normal rats, of which the most common is the so-called alternate (Ab) pattern [7–9,30,32]. After spinal injury, the proportion of steps with this order of limb recruitment diminishes, with a shift toward the other five patterns [7–9,30,32].

The concept of regular step patterns is also the basis of the Regularity Index (RI). This index grades the degree of coordination by computing the proportion of paw placements in normal step sequence patterns during locomotion as a percentage. The RI is 100% if only normal step sequence patterns are used during uninterrupted locomotion, whereas impairment of coordination leads to missteps interspersed between regular step patterns, causing the RI value to decrease [7–9,30,32].

Other indices of locomotion, such as the timing relationships between footfalls [10] and the stride length or step cycle duration, have been used only rarely to analyze forelimb–hindlimb coordination in rats. Low thoracic lesions were found to differentially affect the fore- and hindlimb stride lengths, with the former being shorter than the latter [7,31]. The severity of these symptoms was dependent on the extent of the SCI [7].

Most studies devoted to the problem of forelimb–hindlimb coordination in spinal cord-injured rats have used the CatWalk test to analyze and quantify locomotion. Although this method provides the experimenter with a powerful tool with which to study various aspects of locomotion (for a review see Hamers et al. [8]), it might not be adequate for weaker forms of forelimb–hindlimb coordination impairment, because the number of steps captured during one trial is limited by the 90-cm-long walkway, i.e. to approximately 6 step cycles. Therefore, if a misstep occurs rarely, it may not be recorded and thus the Regularity Index would show no coordinative impairment.

The aim of the present study was, therefore, to analyze forelimb–hindlimb coordination in spinal cord-injured rats during unrestrained locomotion along an extended walkway, enabling the study of a longer sequence of steps. The traditional method of footprint recording [34] was used with the feet colored differently [2,33,35–37]. Rats were divided into three groups according to the extent of their SCI. We describe changes in locomotor speed and fore- and hindlimb step lengths, including the analysis of missteps and the circumstances in which they occur – a problem that has not been analyzed in detail before. The number of missteps, referred to as unpaired forelimb steps, was correlated with differences in the fore- and hindlimb stride lengths. These steps were observed whenever a specific pattern of homolateral limb placement occurred to change the spatial order of paw prints. Their number and duration depended on the extent of SCI. Even in rats that recovered from the injury to attain locomotor speeds similar to preoperative values, with equal fore- and hindlimb stride lengths, the forelimb–hindlimb coordination was changed, as shown by an increase in the distances between a number of fore- and hindlimb footprints.

The aim of the present study was also to analyze changes in the phase shifts between particular limbs during locomotion, the patterns of limb support in different epochs of the step cycle [38,39] as well as their dependence on speed. None of these parameters has so far been analyzed in detail in rats with spinal injuries. We found that the animals showed changes in (1) forelimb–hindlimb coordination, from an almost completely synchronized trot observed before surgery to a lesion-dependent shift for walking, and in (2) the limb recruitment pattern as an adaptation to help them maintain equilibrium during locomotion. These changes partly resembled those observed by us in spinal injured cats [23,25–27], but the deficiencies in rats were both smaller and lasted for a much shorter time.

2. Materials and methods

2.1. Subjects

The experiments were performed on 9 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. The rats were subjected to partial spinal cord lesion and their locomotion along a flat runway was tested before and after surgery. The locomotion of some of these rats, tested 2 or 3 months postsurgery using contact electrodes and EMG recording, has been described previously [38,39]. 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. Experimental procedure

For a 2-week period preceding the experiments, the animals were taught to move at a relatively constant speed along a runway 2.0 m long and 12 cm wide, situated 1.5 m above the floor, using a shelter-seeking paradigm (for details see Górska et al. [5,38,39]). 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^{-1}, respectively.

For footprint recording, the runway was covered with plotting paper (1 mm divisions) and the rats' fore- and hind paws were inked with dyes of different colors [2,35,36], which allowed easy distinction between their prints. An example of footprint recording during runway locomotion is shown in Fig. 1.

For each animal, one experimental session consisted of 10–12 passes along the runway, with 10–14 steps per pass, which gave a sample of approximately 100 steps collected per session for every rat. For the intact animals, data for the final session prior to surgery was used 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. Thereafter, their locomotion was examined every two weeks or once a month on the runway where the rats' performance was found to be better than in the open field [5,9].

Footprint recording was resumed for the majority of rats 13–15 days after surgery, except for 3 animals (S1, M1, L2), which were first tested somewhat earlier (9–11 days) (cf. Table 1). By this time, all rats had regained hindlimb weight support and plantar stepping. Recording was continued once or twice a month for the next 3–3.5 months. At the end of the experiment the rats were sacrificed and their spinal cords taken for histology.

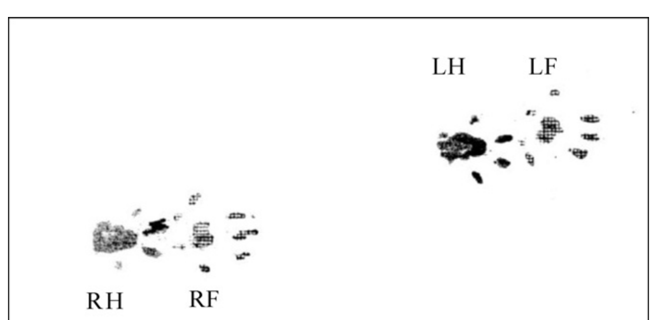

Fig. 1. Examples of rat footprint recording during runway locomotion. L and R – left and right. F and H – fore- and hindlimb.