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Research report

A 3D analysis of fore- and hindlimb motion during locomotion: Comparison of overground and ladder walking in rats

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Abstract

The locomotor pattern, generated by the central pattern generator, is under the dependence of descending and peripheral pathways. The afferent feedback from peripheral receptors allows the animal to correct for disturbances that occur during walking, while supraspinal structures are important for locomotion in demanding situations such as ladder walking. Such walking, by regards to the control needed for accuracy of movements, is now widely used for description of consequences of nervous system dysfunction on motor performance. It is important to have a good knowledge of the changes in kinematic parameters according to walking conditions, since it might reflect different neural mechanisms. The aim of this work was to perform a 3D kinematic analysis of both hind- and forelimb during overground and ladder walking, to study qualitative and quantitative locomotor characteristics in different modes of locomotion. The analysis was performed on 5 rats. Movements of the right hind- and forelimb were evaluated using a 3D optical analyser, and EMG of the soleus and tibialis anterior muscles was synchronously recorded. Results indicate that kinematic and electromyographic characteristics of locomotion are dependent on the type of support. Changes were more obvious for hindlimb than for forelimb. Velocity, stride length and tibialis anterior burst duration were lower on ladder than on runway. In addition, during ladder walking, a protraction was noticed, rats bring their feet more rostral at the end of the swing phase. All these changes constitute an adaptive strategy to allow a better tactile activity with forelimbs and to avoid foot misplacement.

Keywords: Kinematics; Electromyography; Motor cortex; Adaptation; Joint angles; Stance; Overground walking; Horizontal ladder

1. Introduction

Many studies concerning neurological diseases or lesion of some parts of the central or peripheral nervous system are now conducted on rats. The neurological function as well as the effects of various strategies of repair or pharmacological treatments can be assessed by the analysis of locomotion. The kinematic and electromyographic characteristics of locomotion are dependent on various parameters such as the rat strain [30] and the environment. In particular, Pereira et al. [26] have recently shown some subtle impairments of the hindlimb kinematics in treadmill locomotion compared with overground walking. In addition, differences in hindlimb motion are noticed when comparing overground locomotion and walking on a horizontal ladder [5].

The changes in kinematic parameters according to the experimental protocol might reflect different neural mechanisms. The basic locomotor pattern, generated by the so-called "central pattern generator" (CPG) located in the spinal cord, is under the dependence of descending and peripheral pathways [28]. The afferent feedback from peripheral receptors allows the animal to correct for disturbances that occur during walking. On the other hand, supraspinal structures are important for locomotion in demanding situations such as ladder walking, which requires to control the accuracy of movements [2,3]. For instance, the neurons of the forelimb representation of the motor cortex present a higher firing rate during skilled walking, such as walking on a ladder, than during "simple" stepping [2]. In consequence,

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the ladder walking test is now widely used for description of consequences of motor cortex injury [14,23] activity blockade [17], spinal cord injury [5], dopamine depletion [24], ischemia [22] on motor performance. Most of these studies are based on time measurements and quantitative and qualitative evaluation of foot placement accuracy. Recently, Bolton et al. [5] have performed a 2D kinematic analysis, which provided differences in hindlimb position at selected points of the step cycle during ladder walking with respect to runway locomotion. However, to our knowledge, no data is available concerning the forelimb motion during ladder walking.

In this context, it is very important to have a precise knowledge of qualitative and quantitative locomotor characteristics in different modes of locomotion. Thus the aim of our study was to perform a 3D kinematic analysis of both hind- and forelimb during overground walking and ladder walking. Moreover, data obtained in the hindlimb are discussed relative to those obtained in treadmill walking and presented in a previous paper [8].

2. Materials and methods

All procedures described below were approved by both the Agricultural and Forest Ministry and the National Education Ministry (veterinary service of health and animal protection, authorization 59-00999).

2.1. Animals

The experiments were performed on five male Wistar rats weighing approximately 300 g purchased from a commercial breeder (Harlan, France). Animals were housed with a regular light/dark cycle (lights on 07:00–19:00 h) and constant temperature (23 °C). They had free access to water and food.

Two weeks before the experiments, the animals were trained to walk on the apparatus. Three training session were performed. After that, the rats were left undisturbed until recording session. The walking tract was a corridor (length: 1 m; width: 12 cm) made of Plexiglas walls with a floor made of metal rungs (diameter: 4 mm) regularly spaced by 2 cm. The ladder could also be covered with a plastic surface (runway). At the beginning of the training session, the rats were placed on the apparatus and were allowed to explore it for a few minutes. After that, the rat was placed on the corridor at one extremity, and its home cage was positioned under cover to create a familiar environment at the other end.

For the recording session, rats were first tested a maximum of five times on the runway and then five times on the ladder with a 60 s-rest in their home cage between each trial.

2.2. EMG electrodes

Surgical procedure for electrode implantation has already been described in details elsewhere [9]. Briefly, bipolar electrodes were made from two insulated multistranded stainless steel wires (seven strands, 50 μm gauge, AM System, USA). The recording surface was obtained by mechanically removing the insulation for a length of 0.5 mm under a dissecting microscope. The sections of the cut wires were insulated by pulling the Teflon coating over the ends. One pair of electrode wires and a third (common earth) were attached to a six-pin connector (Plastic Products Company, USA) with dental acrylic.

For electrode implantation, the rats were anaesthetized with sodium pentobarbital (60 mg kg^{-1}, ip). The connector was secured to the skull by means of dental acrylic and of three screws. The soleus and tibialis anterior muscles of the right hindlimb were exposed; a pair of electrode wires was inserted into the midbelly of each muscle through a hypodermic needle. A light stimulation (0.5 ms square pulses, 0.5–1 mA) was performed through the electrode wires to verify that they were well localized. Electrodes were then secured by means of sutures (Monosof, Tyco, France) at the entry and exit from the muscle. The common earth was placed in the back of the animal. An antiseptic (Betadine®) was applied on incision areas. Rats were allowed to recover for three days before the first recording session.

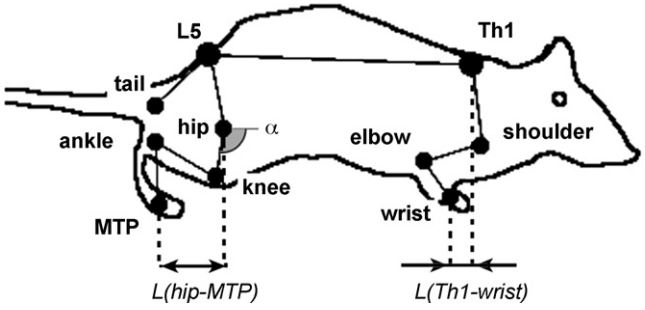

Fig. 1. Diagram showing the position of joint markers. The dashed vertical lines are used to visualize the horizontal distance between hip and MTP markers and between first thoracic vertebra and wrist (respect. $L_{hip–MTP}$ and $L_{Th1–wrist}$ values in Fig. 4C and D). The angle of hip joint (α) was measured with respect to a horizontal line.

2.3. EMG recording and analysis

A flexible cable was used to link the head connector to a low friction rotating connector (Plastic Products Company, USA) positioned above the walking apparatus. This second connector was linked to a differential amplifier (Model 1700, AM System, USA). The raw EMG signal was amplified (1000×, band pass 10 Hz to 10 kHz) and digitized at 1080 Hz. EMG analysis was performed with Spike2 software (Cambridge Electronic Design, UK).

2.4. Kinematic recording and analysis

Movements of the right hindlimb were measured with a 3D-optical analyzer (Vicon). Adhesive infrared-reflective disks (diameter: 0.5 cm) were placed on the shaved skin over eight anatomic landmarks (Fig. 1): the fifth metatarsophalangeal joint (MTP), the lateral malleolus (ankle), the knee, the trochanter major (hip), the tail origin, the wrist, the olecranon (elbow), the acromion (shoulder). In addition, two hemispheric markers (diameter: 1 cm), obtained from a foam rubber sphere cut in two parts and covered by adhesive infrared-reflective paper, were placed between the iliac crests at the 5th lumbar vertebra (L5) and between the scapulae, at the first thoracic vertebra (Th1). In order to minimize variability in marker positioning, all experiments were performed the same day, and all markers were placed by the same operator.

Eight CCD cameras were used to record position of these markers. Calibration was conducted according to Vicon Peak Motion Analysis System proceedings. Kinematic data were collected at a sampling rate of 120 Hz. The directions of lab coordinate *X*-, *Y*- and *Z*-axis were lateral, anterior and vertical, respectively. These cameras covered a 65 cm length of the walking tract, and allowed the recording of 5–7 consecutive steps at regular speed. The coordinates were used to reconstruct the movements in the form of stick diagram or angular joint displacement (Fig. 2). The analysis was performed on at least 10 steps per rat and per condition (ladder or runway). This is above the minimum number of steps (four) that must be recorded to eliminate deviant curves [13]. The steps were chosen from regular walking sequences.

The following parameters were included: step cycle duration and duration of the stance and swing phases; stride length; angular variation of ankle, knee, hip, wrist and elbow. Hip angle was formed by the hip–knee segment relative to a horizontal line. Angular displacements indicated are 3D spatial values.

The moment of the MTP (or wrist) contact was regarded as the beginning of a gait cycle and the next MTP (or wrist) strike of the same leg was regarded as the end of stance. The gait cycle was then split into two parts, the stance and swing phases. The stance duration was defined as the time from paw contact to toe-off, and swing duration from toe-off to the next contact. The stride length corresponds to the distance covered by the paw during the swing phase. Protraction and retraction of the limb at toe-off and at paw contact were evaluated by the horizontal distance between hip and MTP markers ($L_{hip–MTP}$) for hindlimb and between Th1 and wrist markers ($L_{Th1–wrist}$) for forelimb. Quantita-