Journal of Neuroscience Methods 190 (2010) 279–288

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

A semi-automated software tool to study treadmill locomotion in the rat: From experiment videos to statistical gait analysis

P. Gravel[a,*], M. Tremblay[b], H. Leblond[b], S. Rossignol[b], J.A. de Guise[c]

[a] *Département de radiologie, Centre hospitalier de l'Université de Montréal, Québec, Canada*
[b] *Groupe de recherche sur le système nerveux central, Département de physiologie, Université de Montréal, Québec, Canada*
[c] *Laboratoire de recherche en imagerie et orthopédie, Département du génie de la production automatisée, École de Technologie Supérieure, Montréal, Québec, Canada*

ARTICLE INFO

Article history:
Received 28 January 2010
Received in revised form 6 May 2010
Accepted 6 May 2010

Keywords:
Video processing
Robust methods
Bootstrap analysis
Gait patterns
Motion tracking

ABSTRACT

A computer-aided method for the tracking of morphological markers in fluoroscopic images of a rat walking on a treadmill is presented and validated. The markers correspond to bone articulations in a hind leg and are used to define the hip, knee, ankle and metatarsophalangeal joints. The method allows a user to identify, using a computer mouse, about 20% of the marker positions in a video and interpolate their trajectories from frame-to-frame. This results in a seven-fold speed improvement in detecting markers. This also eliminates confusion problems due to legs crossing and blurred images. The video images are corrected for geometric distortions from the X-ray camera, wavelet denoised, to preserve the sharpness of minute bone structures, and contrast enhanced. From those images, the marker positions across video frames are extracted, corrected for rat "solid body" motions on the treadmill, and used to compute the positional and angular gait patterns. Robust Bootstrap estimates of those gait patterns and their prediction and confidence bands are finally generated. The gait patterns are invaluable tools to study the locomotion of healthy animals or the complex process of locomotion recovery in animals with injuries. The method could, in principle, be adapted to analyze the locomotion of other animals as long as a fluoroscopic imager and a treadmill are available.

1. Introduction

In order to study locomotion, especially if one wants to assess deficits in walking after a spinal cord injury, it is crucial to obtain accurate quantitative measures. Traditional methods in locomotion studies consist in filming a subject that has markers fixed over the chosen articulations while walking on a treadmill (Rossignol, 1996). This way, the *X* and *Y* coordinates of each marker can be extracted in order, for instance, to reconstruct the movements or to compute the angular excursions. The use of a treadmill allows the production of a high number of quantifiable step cycles at a desired speed and thus the normalization of the experimental conditions during the tracking of the legs movements. However, when small rodent like mice or rats are used to study locomotion with that paradigm (Leblond et al., 2003), slippage of the skin over the bones and other soft tissues of the limbs prevents having precise and direct measures of joint positions. The major slippage occurs at the level of the knee joint and triangulation is necessary to extract the knee position using hip and ankle joint markers of the femur and tibia (Leblond et al., 2003; Pearson et al., 2005).

The use of cine-radiography allows direct visualization of bone structures during movements (Fischer et al., 2002; Herbin et al., 2007; Vidal et al., 2004). Over the last few years, a technique using a combination of high-speed digital camera and cine-radiography to film animals walking on the treadmill was developed. Extracting the gait pattern data from these sequences is a tedious procedure that involves several stages: image preprocessing, tracking of morphological markers in video frames and gait analysis. Because a fluoroscopic setup is used, the preprocessing stage involves some image distortion correction, denoising and contrast enhancement.

Geometric image distortions caused by the X-ray imaging chain give rise to a variation in magnification across the image field of 5–10% for routinely used image intensifier sizes (Brown et al., 1977; Gronenschild et al., 1994). Such distortion effects are important since a rat can sometime accelerate or decelerate its pace on the treadmill. As a result, its anterior and posterior limbs can come close to the edge of the field of view and result in distorted images. The bones may appear shortened or dilated depending on the type of image distortion (barrel or pincushion) and the measured angles subtended by the articulations are over- or underestimated. The standard way of measuring the distortions relies on the image of a rectilinear calibration grid placed against the input screen of the image intensifier (LeFree et al., 1985; Reiber

* Corresponding author. Tel.: +1 514 890 8000/25877.
E-mail address: pierre.gravel@etsmtl.ca (P. Gravel).

0165-0270/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2010.05.006

et al., 1984). The grid is generally made with horizontal and vertical alignments of bronze ball bearings or with a metallic wire grid pattern encased in a plastic plate (Kooijman et al., 1982; LeFree et al., 1986). A number of semi- or fully-automated computer procedures for the analysis of the calibration grid image were developed to assess the 2D deformation field responsible for the image distortions (Beir et al., 1991; Gronenschild, 1997; Tuzikov et al., 1992). This information can then be used to correct the spatial distortions using the inverse transformation and image interpolation methods.

Since several morphological markers should be tracked on the rat hind leg, it is important to get the best bone/soft tissues image contrast to locate them. Each video frame must be denoised and contrast enhanced for that purpose. Wavelet-based denoising methods (Misiti et al., 2009) perform better than conventional linear filters (e.g. Gaussian smoothing) in preserving image edges while attenuating the noise. In low spatial resolution images, a Gaussian filter may fuse bone articulations by smoothing their edges whereas wavelet denoising may often keep them unaltered. The edge information can then be used to enhance image contrast using an intensity gradient based method.

The manual tracking of morphological markers using a computer mouse is long and tedious. For example, the tracking of six markers in a typical 466-frames video requires positioning the computer mouse about 2800 times. This is a long, painful and often difficult task to accomplish. It takes between 1 and 2 h for a trained user to process a single video sequence. Moreover, treadmill locomotion studies usually require analyzing a few dozen video sequences; even a semi-automated method reducing this time by any factor would be a definite advance in data acquisition.

Gait patterns, reconstructed from the time evolution of 2D morphological markers trajectories across a video sequence, are usually analyzed to estimate the mean gait patterns and associated prediction and confidence bands (Sutherland et al., 1988). There are two different approaches to estimate these bands; the point-by-point Gaussian theory intervals (Natrella, 1963) and the Bootstrap method (Lenhoff et al., 1999; Sutherland et al., 1988). The second one, more precise, is nevertheless sensitive to outliers; the error bands may be overestimated. A more robust Bootstrap method (Cleveland, 1979, 1993) must be implemented.

We present a computer-aided method for the tracking of morphological markers in fluoroscopic images of a rat walking on a treadmill. The method involves: (1) image processing, (2) motion tracking of morphological markers and (3) statistical gait analysis. The imaging protocol used in this work is described in Section 2 as well as the multi-stage implementation of the gait pattern estimation method. Image processing and gait pattern results as well as performance assessment for each stage are presented in Section 3. The discussion and conclusion follow in Section 4.

2. Materials and methods

2.1. General protocol

Adult wistar rats ($n = 6$) weighting 250–300 g were used in this study. The rats were first trained to walk, twice daily, on a motor-driven treadmill belt at a comfortable constant speed for periods of 5 min. Once the rats walked with regularity on the treadmill, small bouts of X-ray videos were recorded as described below. These sequences were then processed and used for analysis and development with our analysis software. The well being of rats was always ensured and all procedures followed a protocol approved by the Ethics Committee at Université de Montréal, according to the Canadian Guide for the Care and Use of Experimental Animals.

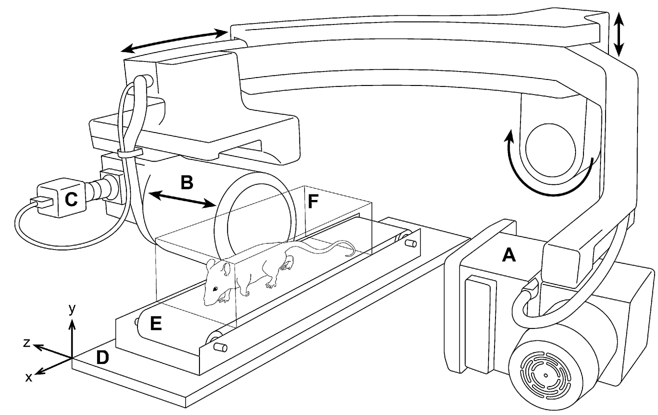

Fig. 1. Experimental setup. (A) X-ray source, (B) image intensifier, (C) high-speed area scan camera, (D) free moving table, (E) variable-speed treadmill, and (F) confinement box.

2.2. Experimental setup

The setup is made of: (1) a Coroskop C arm X-ray system from Siemens, equipped with an image intensifier OPTILUX 27 HD including a field operation diameter adjustable to 27, 17 and 14 cm, (2) a high-speed area scan camera from Dalsa (DS-41-300K0262), equipped with a C-mount zoom lens (FUJINON-TV, H6X12.R, 1:1.2/12.5–75) mounted on the image intensifier, (3) a stand-alone high-speed real-time imaging system software (VisonNow) from Boulder Imaging, supporting the camera's controls, image-capture and streaming to disks, (4) a Plexiglas confinement box, 13 cm × 40 cm × 13 cm, with a removable top covering a variable-speed treadmill and (5) a free moving *X* and *Z* axis table with a motor controlled *Y* axis.

As illustrated in Fig. 1, the treadmill with the overlying box was placed on a free moving table and positioned near the X-ray image intensifier. The X-ray side view videos of locomotion were captured while the animal walked freely at different speeds imposed by the treadmill. The rat was kept in the center of the image intensifier by moving manually the free moving table *X* axis. For 2 and 4 legs X-ray digital video, the image intensifier was set to a diameter of 17 and 27 cm respectively, and the left legs were identified with tin (Sn) rings.

Digital X-ray video frames (512 × 512 pixels with 256 grey levels) were grabbed at 120 frames-per-second with a shutter speed of 2 ms. The slowest and fastest speeds of the treadmill were set at 14 and 30 m/min accordingly. At this frame rate, 12 ± 1 and 25 ± 0.5 images were grabbed at the fastest speed in the swing and stand phases respectively.

From 8 to 20 step cycles were taken from raw digital X-ray videos and converted to AVI uncompressed file for analysis. Depending of the rat performance and treadmill speed, this might represent anywhere between 400 and 2500 frames.

The 2 ms shutter speed was chosen to minimize the drag of the toes in images during the swing phase. At this shutter speed, maximization of the number of gray levels (192/256) in images was obtained: (1) by setting the X-ray output to 100 kVp/16 mAs, (2) by minimizing the distance between the X-ray source and the image intensifier to 82 cm and (3) by setting the C-mount zoom lens at full opening ($F = 1.2$) and maximum focus, and by zooming in all the selected field operation diameter.

Output setting of the X-ray system was conveniently modified electronically to provide fixed values from 40 kVp/0.5 mAs to 100 kVp/16 mAs, linearly with a precision potentiometer, or by blocking the automatic kVp/mAs adjustment once reaching a desired value with a push button.