Journal of Neuroscience Methods 180 (2009) 273–277

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

Quantification of gait in dystonic Gunn rats

Kunal D. Chaniary[a], Mark S. Baron[b,*], Ann C. Rice[b], Paul A. Wetzel[a], Viswanathan Ramakrishnan[c], Steven M. Shapiro[b,d,e,f]

[a] Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23298-0599, USA
[b] Department of Neurology, Virginia Commonwealth University, Richmond, VA 23298-0599, USA
[c] Department of Statistics, Virginia Commonwealth University, Richmond, VA 23298-0599, USA
[d] Department of Pediatrics, Virginia Commonwealth University, Richmond, VA 23298-0599, USA
[e] Department of Physiology, Virginia Commonwealth University, Richmond, VA 23298-0599, USA
[f] Department of Otolaryngology-Head & Neck Surgery, Virginia Commonwealth University, Richmond, VA 23298-0599, USA

ARTICLE INFO

Article history:
Received 12 September 2008
Received in revised form 28 February 2009
Accepted 27 March 2009

Keywords:
Gait
Dystonia
Rat
Hindlimb spread
Step length ratio variability
Kernicterus
Jaundice

ABSTRACT

Spontaneously jaundiced Gunn rats exposed to sulfadimethoxine develop bilirubin encephalopathy (kernicterus) with hearing loss and dystonia, closely resembling the human syndrome. We recently characterized the electromyographic activity in this animal model supporting our clinical impression of dystonia. The objective of this study was to develop a simple, non-invasive method to quantify the motor deficits in dystonic rodents. On postnatal day 16, Gunn rats were treated with 100 mg/kg of sulfadimethoxine or saline. On postnatal day 31, the ventral view of the animals was videotaped while the animals walked inside a Plexiglas chamber. Individual video frames were reviewed and specific gait parameters including hindlimb spread, step length ratio variability, stance/swing ratio and walking speed were compared between dystonic and non-dystonic jaundiced and non-jaundiced littermates. Data analysis demonstrated statistically significant increases in hindlimb spread and step length ratio variability and decreases in walking speed in dystonic animals as compared to controls. This study demonstrates a valuable technique to objectively characterize dystonia in Gunn rats, which could have wide use for other experimental movement disorders as well.

1. Introduction

Dystonia is a movement disorder characterized by patterned, repetitive, and sustained muscle contractions that cause ineffective and often painful movements (Fahn, 1988; Volkmann and Benecke, 2002). Patients often develop distinct walking patterns and balance disturbances (Snijders et al., 2007). Particularly in generalized forms, the disorder may become so severe as to prevent independent standing and walking (Watts and Koller, 2004). The underlying pathophysiology of dystonia is poorly understood and treatments are inadequate (Vitek et al., 1999; Zhuang et al., 2004). Therefore, experimental studies in animal models are critically necessary towards understanding the underlying pathophysiological mechanisms and ultimately developing better therapies (Jinnah et al., 2005).

The jaundiced Gunn rat model is a well-established model of bilirubin encephalopathy and kernicterus (Shapiro and Hecox, 1989). Our group has recently developed procedures to reliably produce sustained mild to moderate generalized dystonia in these animals (Chaniary et al., 2008). Homozygous recessive jaundiced (jj) Gunn rats lack the enzyme uridine diphosphate glucuronosyl transferase, which is immature in human neonates and contributes to physiological jaundice of the newborn (Johnson et al., 1959; Strebel and Odell, 1971). Acute bilirubin encephalopathy is produced in Gunn rats by injecting them with sulfadimethoxine (sulfa) on postnatal day 16 to displace bilirubin from blood albumin binding sites allowing its migration into tissues, including the brain (Schutta and Johnson, 1969; Rose and Wisniewski, 1979). Heterozygous non-jaundiced (Nj) rats have about 50% enzyme activity and are phenotypically normal. Although gait disturbances are seen in jj Gunn rats, gait dynamics in this model have not been described previously.

A number of groups have developed other valuable animal models of dystonia (Raike et al., 2005) and various rating scales have been introduced to assess the dystonia (Richter and Löscher, 1998; Jinnah et al., 2000) in these models. Deficits in genetically dystonic (dt) rats have been assessed by describing abnormal motor features (falls, twists, clasps, pivots) and motor performance (activity, climbing, righting, homing, hanging) (LeDoux et al., 1995). Motor performance of Dyt1 knockdown mice has been successfully mea-

* Corresponding author at: Department of Neurology, Sanger Hall, Virginia Commonwealth University, PO Box 980599, Richmond, VA 23298-0599, USA. Tel.: +1 804 828 7040; fax: +1 804 828 5654.
E-mail address: mbaron@mcvh-vcu.edu (M.S. Baron).

0165-0270/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2009.03.023

sured using rotarod, beam-walking and open-field tests (Dang et al., 2006). Because the principle clinical features of the dystonia vary between animal models, different techniques may be preferable for characterizing the different dystonias. Regardless, novel objective techniques are needed to quantify the dystonias and provide sensitive measures to detect subtle forms of the movement disorder (Jinnah et al., 2005). Gait analysis has been used extensively to quantify experimental spinal cord injuries (Cheng et al., 1997; Metz et al., 2000), peripheral neuropathy (Yu et al., 2001) and Parkinson disease (Amende et al., 2005) in rodent models. In these studies, quantitative aspects of gait have been assessed from video recordings of freely moving animals. The purpose of our study was to adapt current gait assessment techniques to be used for experimental assessment of dystonia in rodents. Based upon visual inspection of Gunn rats, we chose to assess specific gait parameters to highlight the clinical features of dystonia, including hindlimb spread, stance to swing ratio, walking speed and a novel parameter, step length ratio variability. In turn, we anticipated that our findings would be universally utilizable, while being non-invasive and readily interpretable.

2. Materials and methods

All animals and procedures used for the study were approved by the Institutional Animal Care and Use Committee (IACUC) at Virginia Commonwealth University. A total of 32 animals from six different litters were used for this study. The methods and the treatment have been described previously (Chaniary et al., 2008). Briefly, at 16 days of age, 18 jj animals received an intraperitoneal injection of sulfadimethoxine 100 mg/kg. Five of these animals developed mild to moderate motor disability and comprised the experimental group. Of the remaining animals, six were severely affected and did not survive the acute bilirubin encephalopathy, and seven were not affected and were not studied further. The experimental group ($n = 5$) was compared with jj controls given saline ($n = 5$) and Nj controls given either sulfa ($n = 4$) or saline ($n = 4$). Body weights were regularly monitored as an indicator of the general condition of the animal.

2.1. Clinical scores

On postnatal day 15 (baseline) and again on postnatal day 31, dystonia was rated subjectively using a Kernicterus Clinical Rating Scale (KCRS) (Shaia et al., 2002) based on Schutta and Johnson (1969) and Rose and Wisniewski (1979) as follows: 0, normal; 1, slight limb dystonia and gait abnormality; 2, mild limb dystonia and gait abnormality, and impaired righting reflex; 3, moderate limb dystonia and gait abnormality with prolonged righting reflex; 4, severe failure of ambulation, general lack of spontaneous movement with occasional bursts of hyperactivity, and no righting reflex; 5, moribund, including seizures and agonal respiration. An additional 0.5 was added to the score of animals appearing to be midway between one category and the next higher one.

2.2. Gait analysis

The experimental setup was adapted from the design by Yu et al. (2001). Animals were videotaped while spontaneously walking inside a Plexiglas chamber towards a darkened goal box. The rats were trained initially and quickly acclimatized to the experimental conditions. The magnification of the camera was calibrated so as to accommodate a 25 cm reflected ventral view of the chamber. The bottom panel of the chamber was marked with horizontal and vertical grid lines one cm apart. In order to determine the orientation of the body relative to the grid, the sagittal plane of the body on the ventral side was marked with a red marker. This line acted as a body reference marker that was taken into consideration while calculating the gait parameters. In each rat, three satisfactory runs without pauses were recorded for analyses. Digital video images were collected at a rate of 30 frames per second. Video recordings were obtained on postnatal day 15 (baseline) and again on postnatal day 31, 2 weeks after sulfa treatment. To study the locomotor deficits, we analyzed the following gait parameters:

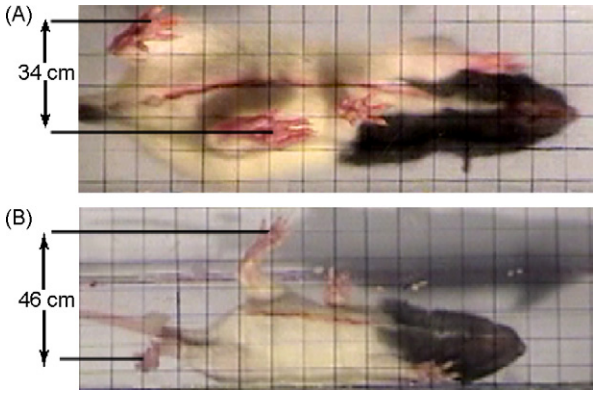

Fig. 1. Ventral view during gait analysis. (A) Normal non-jaundiced (Nj) rat. (B) Abnormal hindlimb spread seen in dystonic rat. Horizontal and vertical grid lines seen in the figure assist with gait measurements. Representative measurement of hindlimb spread in normal and dystonic rat is highlighted in the figure.

1. *Walking speed*: The walking speed during each trial (distance/time) was determined based on the time (number of frames) required to travel a 25 cm track.
2. *Hindlimb spread*: During each foot placement along the grid, parallel lines were drawn to the body reference marker from the middle phalange of hind feet and the perpendicular distance between them was measured as the hindlimb spread. Representative measure of the hindlimb spread is shown in Fig. 1.
3. *Step length ratio* (*SLR*) *variability*: The SLR variability assessed the inconsistency of foot placement over repeated steps. For calculating variability, first the step length was calculated as the anterior-to-posterior distance from the middle phalange of one hindfoot to the other. The SLR was then obtained by dividing the right to the left step length by the left to the right step length. Measures of SLR variability in each group were determined as the coefficient of variation (CV) which was calculated by using the equation: $((\textit{Standard deviation}/\textit{mean}) \times 100)$.
4. *Stance to Swing ratio* (*SSR*): Duration of the stance and the swing phases were determined by analyzing the videos on a frame by frame basis. The stance to swing ratio (SSR) was obtained by dividing the right stance/swing ratio with that of the left (Yu et al., 2001).

The video recorded was digitized on a computer and individual frames of the video were captured using Studio Quickstart (Pinnacle Systems Inc., Mountain View, CA). Further image analysis was carried out using the image analysis software Image J (NIH).

3. Statistics

Statistical analysis was performed by using the JMP® and SAS® software packages (SAS Institute Inc., 2007). Since each animal is repeated in three trials and over time, these observations are correlated. To account for these correlations the repeated measures mixed effects model was used. The mean ± SE reported are the least square means estimated from this model. The standard deviation and the coefficient of variation were calculated for each group to determine the dispersion from the mean. Measures including the