Behavioural Brain Research 225 (2011) 562–573

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

The sensitivity of two-dimensional hindlimb joint kinematics analysis in assessing functional recovery in rats after sciatic nerve crush

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ARTICLE INFO

Article history:
Received 8 March 2011
Received in revised form 8 August 2011
Accepted 13 August 2011
Available online 22 August 2011

Keywords:
Sciatic crush
Walking function
Joint kinematics
Discriminant analysis
Functional assessment
Sensitivity

ABSTRACT

Walking analysis in the rat is increasingly used to assess functional recovery after peripheral nerve injury. Here we assess the sensitivity and specificity of hindlimb joint kinematics measures during the rat gait early after sciatic nerve crush injury (DEN), after twelve weeks of recovery (REINN) and in sham-operated controls (Sham) using discriminant analysis. The analysis addressed gait spatiotemporal variables and hip, knee and ankle angle and angular velocity measures during the entire walking cycle. In DEN animals, changes affected all studied joints plus spatiotemporal parameters of gait. Both the spatiotemporal and ankle kinematics parameters recovered to normality within twelve weeks. At this time point, some hip and knee kinematics values were still abnormal when compared to sham controls. Discriminant models based on hip, knee and ankle kinematics displayed maximal sensitivity to identify DEN animals. However, the discriminant models based on spatiotemporal and ankle kinematics data showed a poor performance when assigning animals to the REINN and Sham groups. Models using hip and knee kinematics during walking showed the best sensitivity to recognize the reinnervated animals. The model construed on the basis of hip joint kinematics was the one combining highest sensitivity with robustness and high specificity. It is concluded that ankle joint kinematics fails in detecting minor functional deficits after long term recovery from sciatic nerve crush and extending the kinematic analysis during walking to the hip and knee joints improves the sensitivity of this functional test.

1. Introduction

Peripheral nerve injuries are usually followed by abnormal nerve regeneration, axonal misrouting, and incorrect reinnervation of the target organs [1,2] and great effort has been placed in developing new nerve treatments and neurorehabilitation strategies, including alternative surgical techniques [3,4], the use of biomaterial and cellular systems [5–7], nerve electrical stimulation [8–10] and different modalities of exercise [11,12]. Valid and reliable functional tests are required to evaluate the effectiveness of different treatment strategies, particularly because functional recovery is considered the main outcome for translating experimental treatment approaches to humans.

Notwithstanding, functional assessment in animal models of peripheral nerve injury is a challenging task. In the rat, the sciatic nerve is responsible for innervating the muscles of the hindleg and the foot [13]. In this animal, video-based biomechanical analysis of ankle joint motion during walking has been shown to be a valid and reliable method to evaluate end-organ reinnervation and functional recovery after sciatic nerve injury [14–16]. Ankle kinematics during walking, in comparison to other functional tests, like the standard sciatic functional index test, is more sensitive to the degree of motion deficits and more closely related to the extent of nerve regeneration in sciatic-injured rats [16]. In addition, two-dimensional (2D) analysis of ankle joint kinematics during walking has been shown to have high day-to-day and inter-observer reliabilities and to be accurate enough to distinguish between animals carrying injuries to the sciatic, tibial or common peroneal nerves [17].

During cyclic movements, like walking, the angle of an individual joint might be represented by a trajectory in respect to time. Separate parameters can then be obtained from such plots by selecting relevant and identifiable events [14,17–19]. Several measures can then be accessed in order to assess individual joint kinematics. This approach has been employed to analyze ankle joint

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doi:10.1016/j.bbr.2011.08.021

kinematics during the stance phase of walking after sciatic nerve injury in rats [5,6,14,15,17,18,20]. Recent studies also analyze ankle kinematics during the swing phase of walking as the sciatic nerve supplies both dorsiflexor and plantarflexor muscles [17,21]. Also, assessing the 2D ankle kinematics during stance and swing phases of walking is a mean to distinguish between injuries to different hindlimb nerves [17].

In addition to measures of ankle angle, data regarding ankle angular velocity during the rat walking has also been reported for sciatic-injured rats [6,15]. The measures of angular velocity were obtained as an attempt to improve the accuracy of ankle kinematics in assessing functional recovery. In fact, there are measures of ankle joint angle that seem to be in disagreement with the stage of recovery post sciatic injury. For instance, we have previously reported that two weeks after sciatic nerve crush (the denervated period) rats walk with unaltered ankle angle at the instant of opposite toe off, when compared to pre-injury [15]. Other inconsistent ankle kinematics results were also reported in this study [15], as well as in other studies [19,21]. These observations raise concerns about the validity of ankle kinematics during the rat gait, or at least of some of its variables, as a mean to assess functional recovery after sciatic nerve injury. Also important, the use of measuring parameters with poor accuracy may result in contradicting data and inconclusive results.

Walking kinematic changes after sciatic nerve crush likely affect the whole hindlimb and not only the ankle joint [22]. Changes in the normal pattern of hip and knee joints movement during gait might accompany those affecting the ankle joint either as a compensatory mechanism for the lack of ankle and foot motion or due to changes in the central control of hindlimb motion triggered by motor and proprioceptive deficits of the muscles supplied by the sciatic nerve [23]. Therefore, it is possible that assessing hip and knee kinematics will provide extra sensitivity and specificity to walking analysis in the rat sciatic nerve model. To our knowledge data regarding individual kinematics of the hip and knee joints during gait after sciatic nerve injury have not been yet reported.

The sensitivity (i.e. the true positive rate) and specificity (i.e. the true negative rate) are two key properties of diagnostic and screening tests. These test properties can be evaluated using linear discriminant analysis particularly in the case of multivariate tests combining several parameters [24]. Discriminant analysis simply addresses classification or discrimination in which objects or patterns are classified into one of several distinct populations using a predictive model developed on a set of independent variables [25]. The number of subjects that are correctly classified by the discriminant model provides a measure of the sensitivity of the set of testing variables used in its building. Conversely, the number of subjects misclassified is a measure of the specificity of the test variables. Additionally, discriminant analysis gives information about the relative importance of each of the independent variables in classifying the cases, yielding a sound basis for discarding variables with little contribution in separating the groups. Therefore, discriminant analysis might be used to identify which are the best walking joint kinematic parameters to assess functional recovery after sciatic nerve injury in the rat.

In this study we conduct a detailed 2D biomechanical analysis of the hip, knee and ankle joints in the rat and employed linear discriminant analysis to assess the sensitivity and specificity of a large set of spatiotemporal and joint kinematic variables as a test of movement dysfunction after sciatic nerve injury. Sciatic nerve-crushed rats in the denervated (one week post injury) and in the reinnervated (twelve weeks post injury) phases were used as they may serve as a model of animals with severe and minor walking deficits, respectively. Sham-operated controls were used as the normal walking group. We predict that using hip and knee kinematics will improve the sensitivity and specificity of walking analysis to reveal minor functional deficits in sciatic nerve-regenerated rats.

2. Methods and materials

Twenty-four male Sprague-Dawley rats (Harlan Laboratories, Udine, Italy) were randomly assigned to one of three groups. A crush injury to the right sciatic nerve was induced in animals in two of the groups, with animals of the third group undertaking a sham surgery (Sham). Walking tests were performed one week after sciatic nerve injury, a period of complete denervation and paralysis of sciatic-innervated muscles (DEN group) [26], or twelve weeks after either sciatic nerve crush (REINN group) or sham surgery (Sham group). After twelve weeks recovery from sciatic nerve crush, reinnervation and functional recovery are considered as complete, based on results of the standard sciatic functional index [15,16,27]. As a result of the longer follow up time, mean (±SD) weight was higher in the REINN (454.1 ± 15.8 g) and Sham (435.5 ± 17.6 g) groups, compared to the DEN group (351 ± 5.1 g). All procedures were performed with the approval of the Veterinary Authorities of Portugal in accordance with the European Communities Council Directive of November 1986 (86/609/EEC).

The sciatic crush injury procedure was described in detail elsewhere [15]. Briefly, animals were anaesthetized (ketamine 9 mg/100 g; xylazine 1.25 mg/100 g, i.p.) and the right sciatic nerve was exposed by skin incision and by splitting the fascia and muscles overlying the nerve. The right sciatic nerve was crushed in the midgluteal region, and above the point this nerve divides into its terminal branches. Sciatic nerve crush was induced by applying constant pressure for 30 s using a non-serrated clamp [16,28]. The muscle, fascia, and skin were closed with 4/0 resorbable sutures. To prevent autotomy, a deterrent substance was applied to the rats' right hindleg and foot [29,30]. The animals were intensively examined for signs of autotomy and contracture and all animals remained free of severe wounds (absence of a part of the foot or severe infection) or contractures during the study. In sham-operated animals, skin and muscle incisions were performed and the sciatic nerve exposed and mobilized using an identical procedure followed in the other groups, but the sciatic nerve was left intact. All animals were left to recover in their cages with no other measures taken that could enhance nerve regeneration and/or functional recovery.

2.1. Kinematic analysis

An optoelectronic system with six infrared high-speed cameras (Oqus-300, Qualisys, Sweden, frame rate 200 Hz) was used to record the motion of the right hindlimb of walking rats. After shaving, seven reflective markers with 2 mm diameter were attached to the skin of the right hindlimb at the following bony prominences (Fig. 1A): (1) tip of fourth toe, (2) head of fifth metatarsal, (3) lateral malleolus, (4) lateral knee joint, (5) trochanter major, (6) anterior superior iliac spine, and (7) ischial tuberosity. All markers were placed by the same person. Animals walked on a Perspex track with length, width and height of respectively 120, 12 and 15 cm with two darkened cages placed at both ends of the corridor to attract the animals and facilitate walking. Before data collection, all animals performed two to three conditioning trials at separate days to be familiarized with the corridor. During the experimental trials, animals walk at voluntarily speed. Cameras were positioned to minimize light reflection artifacts and to allow recording 4–5 consecutive walking cycles, defined as the time between two consecutive initial ground contacts of the right fourth toe. The motion capture space was calibrated regularly using a fixed set of markers and a wand of known length (20 cm) moved randomly across the recorded field. Calibration was accepted if the standard deviation of the wand's length measures was below 0.4 mm. Planar motion in the sagittal plane of the hip, knee and ankle joint was calculated with Visual 3D software (C-Motion, Inc, Germantown, USA) by a computational procedure implementing the dot product between the skeletal segments articulated by these joints (Fig. 1B). Joint velocity was also calculated using the first derivative of joint angle motion. The trajectory of the reflective markers was smoothed using a Butterworth low-pass filter with a 6 Hz cut-off. Each walking cycle was manually defined by using the linear horizontal velocity of the fourth toe marker, and selecting the time interval delimited by two consecutive initial paw contact events, corresponding to the time point this marker velocity reaches zero (i.e. swing-to-stance transition point). The walking cycles were time normalized by interpolation using the longest trial and an average walking cycle was thereafter calculated per each animal using six individual walking cycles. The hip joint angle was defined as the angle oriented rostrally between the pelvis and femur. Pelvis orientation was defined by the line joining the markers at the anterior superior iliac spine and ischial tuberosity, while femur orientation was given by the segment between markers at the greater trochanter and lateral aspect of the knee (Fig. 1B). Knee angle was defined by the angle oriented caudally between the femur and the leg, with the latter construed by the markers at the knee and lateral malleolus (Fig. 1B). Last, the ankle joint was defined as the angle formed between the leg and hindfoot, with the latter segment defined by markers at the lateral malleolus and head of fifth metatarsal (Fig. 1B). The ankle's zero degrees position corresponded to a straight angle between the leg and hindfoot. Ankle dorsiflexion and plantarflexion angles were then measured as positive and negative angles, respectively. Accordingly, positive angular velocity indicates increasing angle values (i.e. hip and knee extension; ankle dorsiflexion).