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Research report

Evaluation of gait impairment in mice subjected to craniotomy and traumatic brain injury

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HIGHLIGHTS

- DigiGait treadmill analysis was used to study gait changes after brain trauma.
- Stride frequency and duration, and swing were significantly altered.
- Craniotomised control mice also show changes in gait following brain trauma.

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ABSTRACT

Traumatic brain injury (TBI) represents a significant global health burden and causes long-lasting neuromotor deficits, particularly in individuals who sustain severe TBI. A better understanding of gait impairment after experimental TBI will provide valuable information for the recovery and rehabilitation of TBI survivors. Here we utilised the DigiGait system to perform kinematic gait analysis in mice subjected to brain injury induced by the controlled cortical impact (CCI) TBI model. Naïve mice, non-craniotomised and craniotomised mice were included as controls. The temporal and spatial profile of gait was mapped from 3 h to 1-week post-TBI. Remarkably, there was a noticeable alteration in some aspects of gait in craniotomised sham mice from their pre-surgery baseline at various time-points over the testing period. This was not observed in naïve mice or non-craniotomised sham controls over the same time period. This finding indicates that the craniotomy procedure alone effects gait. When craniotomised mice were subjected to TBI, additional deleterious effects on gait function were observed, including forelimb stance and swing duration as well as left hindlimb swing and stride duration and frequency. Hence, mice subjected to CCI-induced TBI develop clear alterations in gait but part of this is attributable to the effect of craniotomy alone. This study also highlights the need to include both non-craniotomised and craniotomised sham mice as controls when undertaking the CCI-induced model of TBI, particularly when early time points are being evaluated.

1. Introduction

Traumatic brain injury (TBI) is caused by physical impact to the head resulting in rapid tissue deformation, and affects 10 million people annually. It is the leading cause of death and disability in children and young adults [1]. In particular, people who sustain TBI show significant gait abnormalities that restrict their mobility and affect their quality of life. Hence there is a need to understand how TBI results in biomechanical gait abnormalities [2]. While the cellular and molecular mechanisms triggered in the brain after experimental trauma in rodents have been extensively researched, few papers have described kinematic gait analysis in rodent TBI models.

Historically, methods for studying gait changes in rodents have been limited to the beam walk test [3], the ladder beam test [4], and the rotarod apparatus [5] which measure combined forelimb/hindlimb dysfunction. The gridwalk system and the spontaneous forelimb test (cylinder test) were subsequently developed to assess motor deficits in individual limbs [6]. These techniques only enable the assessment of few, specific gait abnormalities [6] and are influenced by the animal's stamina, motivation and attention span.

Automated systems of gait analyses have since been introduced, thereby enabling the kinematic analysis of gait which is

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more sensitive than tests reliant on visual observation. These include the CatWalk system (Noldus Information Technology, The Netherlands), the ExerGait (XL) treadmill (Columbus Instruments, USA) which is used in conjunction with the TreadScan® software system (CleverSys, Inc., USA) and the DigiGait treadmill gait analysis system (Mouse Specifics Inc., USA). Each of these devices has been used to study gait patterns in rodents subjected to spinal cord injury [7–9]. The CatWalk system consists of a longitudinal glass platform which is illuminated, and on which rodents are allowed to traverse freely. A camera records their movement and images of the plantar surface of the animals' paws are analysed using specialised software. To date, sensorimotor impairment in rodent models of single TBI has only been thoroughly investigated using the CatWalk system [10]. Recent publications have revealed that the craniotomy procedure, often utilised as a control procedure for the controlled cortical impact (CCI) model of TBI, can produce inflammation [11] and gross neurological dysfunction [12]. Yet, there have been no reports of fine changes in motor function following craniotomy alone.

DigiGait consists of a motorised transparent treadmill, fitted with a digital camera that records the underside of the animals walking on the treadmill belt. Digital paw prints of each of the four limbs are then assessed by proprietary software. Animals may also be challenged to walk up an incline or down a decline. This allows the detection of subtle disturbances in gait that might not be obvious when walking on an even plane. The DigiGait is currently the most widely published ventral plane videography apparatus for gait analysis in laboratory animals. We have previously utilised the DigiGait apparatus to assess gait patterns in genetically modified mice [13]. We have also reported the utility of the DigiGait apparatus to study improvement in gait after administration of certain therapeutic agents in mice subjected to CCI-TBI [14,15]. These studies were limited to a 1 or 1.5 mm impact depth TBI and mice were tested at single, early time-points post-injury and were not compared with naïve controls. Here we used the DigiGait system to assess gait patterns over a one-week period in wildtype naïve mice, non-craniotomised shams as well as craniotomised and CCI-induced TBI at a 2 mm impact depth.

2. Materials and methods

2.1. Mice

All animal procedures were undertaken in accordance with the National Health and Medical Research Council (NH&MRC) Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia. Experiments were performed with adult C57/Bl6-J male mice aged 8–10 weeks which were sourced from the Animal Resources Centre in Perth, Australia. Experiments were approved by the Alfred Medical Research Education Precinct (AMREP) Animal Ethics Committee.

2.2. Controlled cortical impact (CCI) model of traumatic brain injury (TBI)

TBI was induced using the electromagnetic CCI model [16]. Briefly, following anaesthesia with 2,2-tribromoethanol (0.5 g/kg; via intra-peritoneal injection) mice were placed in a stereotaxic frame (Kopf, Tujunga, CA). For preparation of the anaesthetic, a 100% solution of 2,2-tribromoethanol was prepared by dissolving 5 mg in 5 ml *tert*-amyl alcohol. Aliquots were prepared and stored in the dark at −80 °C. For TBI/sham surgeries, fresh anaesthetic solution was prepared by adding 75 μl of 100% 2,2-tribromoethanol to a tube containing 4 ml of pre-warmed saline (final concentration of 1.875%). The solution was mixed by vortexing and the tube was covered with aluminium foil to prevent exposure to light. The skull was exposed following a surgical incision, and a 3 mm diameter circular craniotomy was performed over the left parietal cortex using an electric drill, with the centre at coordinates AP = −2.0, ML = +2.0 from bregma. A drop of water was then applied to the residual bone debris was gently wiped with a cotton swab. To minimise heating during the drilling, the drill tip was placed on ice immediately prior to drilling and also re-cooled at approximately 15-s intervals. The impactor was positioned at an angle of 20° to the dural surface in contact with the dura, and a cortical impact was initiated through the graphical user interface of the software (LinMot, Switzerland) that controlled the CCI device. TBI was induced at an impact depth of 2 mm, with a velocity of 5 m/s and a dwell time of 150 ms. The exposed site was then covered with bone wax, the scalp was sutured and the animals were allowed to recover on a 37 °C heat pad. Sham animals were subjected to craniotomy but not TBI. Non-craniotomised sham mice were subjected to anaesthesia and incision/suture only. Naïve mice were not subjected to anaesthesia, craniotomy or controlled cortical impact.

2.3. Gait analysis

Treadmill gait analysis was performed using the DigiGait™ system (Mouse Specifics Inc., USA). Digital images of paw placement were recorded through a clear treadmill from the ventral plane of the animal. Mice were first tested in a single session prior to injury at a 15 cm s^{-1} treadmill speed. At 3 h, and then at 1, 2, 3, and 7 days post-injury/craniotomy, mice were tested again at the same speed. Naïve and non-craniotomised mice were tested separately at the same time points. For each mouse, videos of ~5 sd duration of all sessions were analysed using the DigiGait™ Imaging and Analysis software v 12.2 (Mouse Specifics Inc., USA). Before each test mice were allowed to acclimatise in the Perspex chamber for 1 min. The treadmill belt and the encasing Perspex chamber were cleaned with 80% (v/v) ethanol in between tests.

2.4. Statistical analysis

We studied $n = 12$ animals for the 2 mm sham/TBI and naïve groups, and $n = 6$ animals for non-craniotomised group. Values greater or less than 2 standard deviations from the average of each cohort were excluded as outliers. This amounted to between 0 and 2 animals per cohort being excluded. To examine temporal changes in naïve mouse gait over the testing period, a one-way repeated measures ANOVA test with Fishers LSD post hoc analysis was carried out across each time point for each paw. Only sham vs. 2 mm impact depth TBI were statistically compared as naïve and non-craniotomised mice were tested separately. Two-way ANOVA with Holms–Sidak post hoc analysis was used to analyse the effect of injury/craniotomy relative to naïve as well as non-craniotomised controls vs. time in each gait parameter for each paw. GraphPad Prism software for Windows, version 6.01 was used for analyses and a p value of <0.05 was considered significant.

3. Results

Mice were subjected to craniotomy or TBI at an impact depth of 2 mm ($n = 10$–12) and assessed from 3 h to 168 h (7 days). We maintained the treadmill speed at 15 cm s^{-1} as mice available to us were unable to walk properly at higher speeds, particularly after being subjected to TBI. For all parameters, no significant differences were noted in sham and TBI groups at $t = 0$ (pre-surgery). Variants of stride in the left forelimb (LF) and left hindlimb (LH) were significantly affected by TBI sustained at the 2 mm impact depth. The swing duration of all four limbs was effected by TBI, and stance duration in the right forelimb (RF) was also altered. Gait patterns