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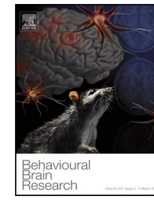

Research report

Quantitative gait analysis of long-term locomotion deficits in classical unilateral striatal intracerebral hemorrhage rat model

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HIGHLIGHTS

- We are the first to perform gait analysis on unilateral intracerebral hemorrhage (ICH) rat models.
- We examine long-term gait disturbance in unilateral ICH rats.
- The parameters of print area, stance time, paw pressure, stride length, base of support, and support on style all exhibit change in hemiplegic rats.
- Asymmetry in print area, stance time, and pressure in the contralateral paws of ICH rats lasts for more than one month.
- Brain tissue loss after ICH is significantly related to gait patterns asymmetry.

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ABSTRACT

Gait analysis is a systematic collection of quantitative information on bodily movements during locomotion. Gait analysis has been employed clinically in stroke patients for their rehabilitation planning. In animal studies, gait analysis has been employed for the assessment of their locomotive disturbances in ischemic stroke, spinal cord injury and Parkinson's disease. The aims of the work reported here were to identify the gait parameters, collected from the computer-generated CatWalk System, that change after unilateral intracerebral hemorrhage (ICH) in the acute stage and long term up to 56 days post-ICH. The results showed that with the collagenase-induced unilateral striatal lesion, the rats displayed a significant contralateral decrease in print and maximum contact area and paw intensity, a diagonal increase in the stance duration of the left front and right hind paws, a significant decrease in the stride length of all four limbs, and foot pattern instability as reflected by the base of support, support on styles, and cadence. These deficits, including those in print area, stance and pressure, were demonstrated throughout the long-term period following ICH. The correlations between the gait parameters, lesion volume and asymmetrical forelimb use were also reported in this paper. This work has provided a systematic description on gait parameters in the classical striatal ICH model, which might become an essential assessment tool in future studies of pathophysiology and the development of novel treatments for experimental unilateral intracerebral hemorrhage with gait deficits.

1. Introduction

Spontaneous intracerebral hemorrhage (ICH) is the most common stroke subtype. It is associated with a high mortality rate and only around half of all survivors are able to maintain independence [1,2]. ICH occurs in select locations of the brain, such as the basal ganglia, cerebellum, and brain stem, resulting in hematoma within the brain parenchyma that triggers a series of events leading to severe neurological deficits, including hemiplegia and hemidysesthesia. Its high rate of long-term disability and lack of pharmacological therapies make ICH one of the world's major health burdens. Many novel strategies that may benefit

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ICH patients have been explored in preclinical research, and several studies designed to mimic human ICH as closely as possible have been carried out to identify potential neuroprotective therapies. The development of appropriate animal models and sensitive evaluations of ICH motor deficits remains an active area of preclinical research into the effectiveness of therapeutic interventions [3,4].

A number of behavioral tests have been developed to determine the motor function outcomes of central nervous system (CNS) diseases in rodent models [5,6]. Existing tests to measure unilateral brain injury include the forelimb placing test, reaching test, cylinder test measuring forelimb use asymmetry, Modified Neurologic Severity Score (mNSS), and corner turn test [6,7]. These tests have been used for decades in studies of sensorimotor function following CNS injury. However, they suffer a number of limitations when used to assess unilateral dysfunction. For example, the forelimb placing test and mNSS require that rats be held by their torsos, while the cylinder test measures only forelimb use asymmetry, with the hind limbs neglected. Few studies have examined asymmetrical sensorimotor deficits in animals with unilateral ICH.

ICH patients may suffer a permanent loss of brain function or long-term deficits that take years to recover from. The main aim of preclinical research is to improve functional recovery in human beings. Most of the ICH animal models in current use are designed to mimic clinical situations. Although deficits may present close to ICH induction, animals that survive the initial ICH usually recover almost completely in approximately two months, with residual motor deficits minimally detectable using current investigative techniques [8,9]. Few studies have assessed functional impairments in long term. In a study using a collagenase model, the neurological deficit score had returned to normal in four weeks [10], and, in other studies, paw placement, cylinder, and ladder tests revealed impairment in one month after ICH [6,11,12]. The reaching test is effective for assessments in six weeks [13]. There is clearly a need for sensitive reproducible behavioral tests that can be used in experimental ICH research to assess the degree of damage and long-term residual impairment caused by unilateral CNS disorders and the process of recovery.

The CatWalk method is an automated, computerized gait analysis technique that allows objective quantification of multiple static and dynamic gait parameters. It has been used to assess gait in animal models of spinal cord injury [14], arthritis [15], pain [16], sciatic nerve injury [17], Parkinson's disease (PD) [18], and ischemic stroke [19], but not yet in animal models of ICH stroke. Hence, there is no experimental evidence concerning the CatWalk system's suitability for this particular application.

The aims of the work reported herein were thus to perform exploratory analysis of gait parameters to identify those that change in ICH rat models and to investigate the long-term deficits displayed by ICH rats using gait analysis. This work is the first to evaluate neurological disorders in ICH rat models using automated gait analysis.

2. Materials and methods

2.1. Animal preparation and intracerebral infusion

Our animal use protocols were approved by the Guide for the Animal Care and Use Committee of the Chinese University of Hong Kong. Forty adult male Sprague-Dawley rats (250–300 g) were used. All of the animals were housed under a 12 h light/12 h dark cycle with free access to food and water.

The rats were anesthetized with the intraperitoneal administration of 0.2 mL/100 g of a mixed solution containing 5 mg/mL of ketamine and 2.5 mg/mL of xylazine, and then secured prone onto a stereotaxic frame before making an incision over the scalp. We then used the stereotactic coordinates to locate the striatum in the right hemisphere: 0.2 mm anterior, 3.0 mm lateral to the bregma and 6.0 mm deep. We drilled a 1-mm borehole, and then inserted a 26-gauge needle. Type IV collagenase (1.2 μL, Sigma, C5138 0.25 U in 1 μL NaCl 0.9%) was infused using a microinfusion pump. The syringe was left in place for 10 minutes before withdrawal to prevent back-leakage before being withdrawn. The borehole was then sealed with dental cement, the incision was closed, and the animals were kept warm and allowed to recover. The animals' body weights and the normality of their ambulation, feeding and grooming were the criteria used in assessing their wellbeing.

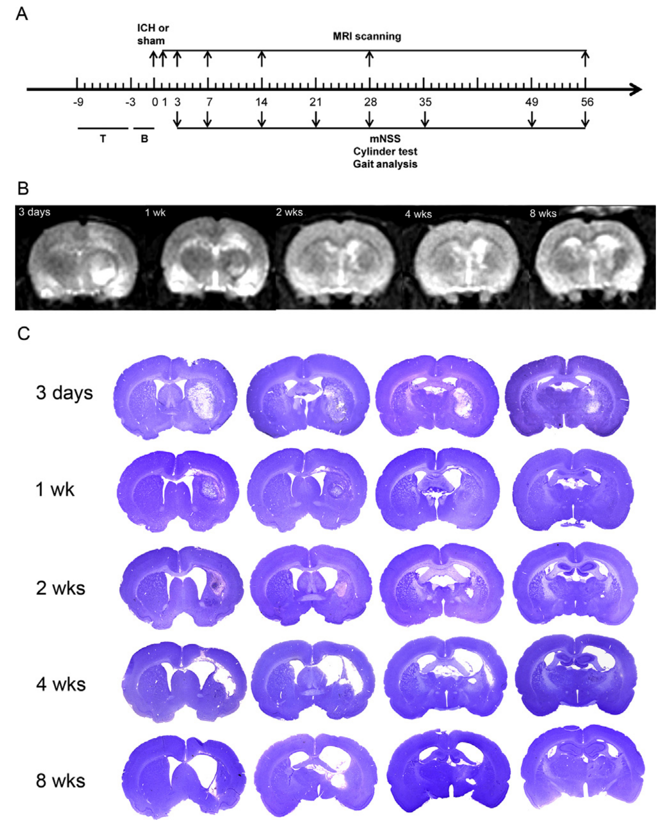

Fig. 1. (A) Schema illustrating the time course of testing procedures. Numbers indicate days relative to the ICH or sham surgery. T: training; B: baseline evaluations. (B) A representative images from MRI scanning at the level of maximum hematoma diameter on day 3, 7, 14, 28 and 56 following ICH. T2W1 of corresponding regions are shown. (C) A representative Nissl-stained image on day 3, 7, 14, 28 and 56 of the ICH rats with a cross section through the entire brain.

2.2. Neurological outcome measurements

Throughout the experiment, the animals were not subjected to any undue stress or irritation, and their condition and wellbeing were monitored. All of the behavioral training and testing (the protocols of which are described in the following paragraphs) were carried out in a quiet room at a fixed time during the animals' light phase by at least two experimenters who were blinded to experimental group. Baseline evaluations were conducted two days prior to surgery. Fig. 1A illustrates the time course of all neurological outcome measurements, with the time points of the testing procedures described in the figure legend.

2.2.1. Modified neurologic severity scores (mNSS)

The mNSS was used to assess the neurologic deficits of the ICH rats from day 1 to day 56 [7,20]. The score is a composite of