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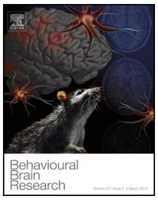

Research report

# Correlation between subacute sensorimotor deficits and brain water content after surgical brain injury in rats

Devin W. McBride[a], Yuechun Wang[a,b,1], Prativa Sherchan[a], Jiping Tang[a], John H. Zhang[a,c,d,*]

[a] Department of Physiology & Pharmacology, Loma Linda University School of Medicine, Loma Linda, CA, USA
[b] Department of Physiology, Jinan University School of Medicine, Guangzhou, GuangDong, China
[c] Department of Neurosurgery, Loma Linda University School of Medicine, Loma Linda, CA, USA
[d] Department of Anesthesiology, Loma Linda University School of Medicine, Loma Linda, CA, USA

## HIGHLIGHTS

- SBI results in significant acute sensorimotor deficits.
- SBI functional deficits correlate with frontal lobe brain water content.
- Beam walking, corner turn, beam balance, and foot fault tests are recommended.

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## ABSTRACT

Brain edema is a major contributor to poor outcome and reduced quality of life after surgical brain injury (SBI). Although SBI pathophysiology is well-known, the correlation between cerebral edema and neurological deficits has not been thoroughly examined in the rat model of SBI. Thus, the purpose of this study was to determine the correlation between brain edema and deficits in standard sensorimotor neurobehavior tests for rats subjected to SBI. Sixty male Sprague–Dawley rats were subjected to either sham surgery or surgical brain injury via partial frontal lobectomy. All animals were tested for neurological deficits 24 post-SBI and fourteen were also tested 72 h after surgery using seven common behavior tests: modified Garcia neuroscore (Neuroscore), beam walking, corner turn test, forelimb placement test, adhesive removal test, beam balance test, and foot fault test. After assessing the functional outcome, animals were euthanized for brain water content measurement. Surgical brain injury resulted in significantly elevated frontal lobe brain water content 24 and 72 h after surgery compared to that of sham animals. In all behavior tests, significance was observed between sham and SBI animals. However, a correlation between brain water content and functional outcome was observed for all tests except Neuroscore. The selection of behavior tests is critical to determine the effectiveness of therapeutics. Based on this study's results, we recommend using beam walking, the corner turn test, the beam balance test, and the foot fault test since correlations with brain water content were observed at both 24 and 72 h post-SBI.

## 1. Introduction

Approximately 1 million neurosurgery cases are performed annually in the United States, and regardless of advances in technology, medical practices, and therapeutics, a significant burden remains on the U.S. healthcare system [1]. Thus, despite how carefully a neurosurgical procedure is performed, it is inherently linked to post-operative complications caused by direct trauma, hemorrhage, and brain edema, resulting in delayed healing, and increased morbidity and mortality [2–5]. This unavoidable injury to healthy tissue which accompanies neurosurgical procedures is termed surgical brain injury (SBI).

Cerebral edema, and subsequent brain swelling, is one of the major post-operative injuries following SBI and significant contributor to patient morbidity, limiting patient recovery and outcomes.

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[*] Corresponding author at: Department of Physiology & Pharmacology, Loma Linda University School of Medicine, Loma Linda, CA 92350, USA. Tel.: +1 909 558 4723; fax: +1 909 558 0119.
*E-mail address:* jhzhang@llu.edu (J.H. Zhang).
[1] Co-first author.

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Several interventions and adjunct treatments are available and utilized to minimize secondary injury after SBI and increase patient recovery rates and outcomes, yet the burden of patient recovery and morbidity remains.

Numerous promising therapies are being explored to manage brain water content and reduce cerebral edema, but their translation from the laboratory setting to clinical practice is hindered, in part by the choice of neurological assessment after experimental SBI [6]. Although therapies which are shown to correspond to reduced brain water content are beneficial, it is the effectiveness of treatments for improving functional outcome and quality of life which is the primary driving force for clinical use.

Choosing the appropriate neurofunctional tests in experimental models depends on the pathology studied; SBI is a unilateral injury which significantly primarily impairs sensorimotor function [5,7–9]. Although a vast number of behavioral tests exist to assess functional recovery after injury [10–13], many of them have not been examined in the SBI rodent model. Additionally, the neurological tests which have been used after experimental SBI have not been investigated for correlation with the severity of injury. Therefore, the primary purpose of this study is to identify correlation between cerebral edema and functional outcome after SBI in rats. The secondary purpose is to evaluate neurological tests which have not previously been examined after SBI and determine their use for functional assessment post-SBI. Finally, a recommendation is made on the most appropriate neurological tests for studying the effects of therapeutics on functional outcome after SBI in rats.

## 2. Material and methods

All experiments were conducted in compliance with the *NIH Guidelines for the Use of Animals in Neuroscience Research* and approved by the Institutional Animal Care and Use Committee at Loma Linda University. Male Sprague–Dawley rats (300–350 g) were housed in a temperature and humidity controlled environment with a 12-h light:12-h dark cycle and given ad libitum access to food and water. Sixty male Sprague–Dawley rats were subjected to either sham surgery ($n=8$) or surgical brain injury ($n=52$) via partial frontal lobectomy (Fig. 1A).

### 2.1. Surgical brain injury

The rodent model of SBI was used as previously described [5]. Rats were anesthetized with isoflurane (4% induction, 3% sustained) delivered in a mixture of medical gas (0.7 L/min) and 99.7% oxygen (0.3 L/min). Animals were then positioned prone and secured onto a standard rodent stereotactic frame. Next, the scalp was incised and a square cranial window was made in the right frontal bone, along the sagittal and coronal planes, 2 mm lateral from the sagittal suture and 1 mm anterior to the coronal suture. The dura was incised and reflected to expose the right frontal lobe. A blade was used to resect the exposed tissue. Hemostasis was achieved using intraoperative packing and saline irrigation. Upon hemostasis, the skin was sutured and buprenorphine (0.01 mg/kg, subcutaneously) and saline (1 mL, subcutaneously) were administered. Sham surgery included only the craniectomy procedure. Total surgery time was between 30 and 50 min.

### 2.2. Neurological evaluations

Neurofunctional tests were conducted at 24 or 72 h post-ictus and analyzed in a blinded fashion.

#### 2.2.1. Composite neuroscore

Composite neuroscore, developed by Garcia et al. [14] for neurological assessment in rats subjected to middle cerebral artery occlusion, was modified for use in rats after SBI. The composite examination consists of seven independent sub-tests which evaluate spontaneous activity, body proprioception, vibrissae touch, limb symmetry, lateral turning, forelimb outstretching, and climbing. For spontaneous activity (SA), the animal was observed for 5 min in a novel environment (cage). For body proprioception (BP), the animal's trunk was stimulated using a cotton swap. For vibrissae touch (VT), a cotton swab, moving from the rear of the animal towards its head, was used to gently touch vibrissae. For limb symmetry (LS), the animal was suspended by the tail to assess movement of the forelimbs. For lateral turning (LT), the animal was placed on a surface and allowed to roam. For forelimb outstretching (FO), the animal was elevated by its tail allowing both forepaws to touch a flat surface (Table 1). For climbing (CL), the animal was placed on a gripping surface (20 cm × 42 cm) aligned in a 45° angle and elevated 36 cm from the surface of a table. The individual test scores are summed to yield the final composite neuroscore for each animal. The minimum score is 3 (worst performance) and the maximum is 21 (best performance).

#### 2.2.2. Beam walking

The animal's ability to traverse a beam was assessed in the beam walking test using an elevated horizontal rod (90 cm × 2.5 cm, 30 cm above the table surface) connected to two platforms [6]. Animals were placed on both platforms for 15 s then placed perpendicularly on the beam halfway between the platforms and given 40 s for testing. Due to the innate tendency to avoid falling, animals walked along the beam towards a platform. Scoring: 5, animal reached the platform within 25 s; 4, animal reached the platform between 25 and 40 s; 3, animal moved halfway to a platform and stayed on the beam for at least 25 s; 2, animal moved less than halfway on the beam and stayed on the beam for at least 25 s; 1, animal did not move and stayed on the beam for 40 s; and 0, animal fell off the beam in less than 25 s if animal moved along the beam or animal fell off the beam in less than 40 s if animal did not move.

#### 2.2.3. Corner turn test

The corner turn test was conducted as previously described [15]. Animals were approached a 30° corner made with two Plexiglas walls. Animals had to rear and then turn either to the left or to the right to exit. Twenty trials were performed (30 s between each trial), and the number of left and right turns were recorded. The number of left turns were expressed as a percentage of the total number of turns, or [((left turns)/(total turns)) × 100].

#### 2.2.4. Forelimb placement test

The forelimb placement test examined the animal's ability to respond to a vibrissae-elicited excitation by extending its forelimb as previously described [15]. Animals were held by their trunk and positioned parallel to a table top. Animals were slowly moved up and down so that the vibrissae of one side were stimulated by the table edge for 20 times on each side. Reactive forelimb placements were recorded. The number left limb placements were expressed as a percentage of the total number of trials (20), or [((left forelimb placements)/(total number of trials)) × 100].

#### 2.2.5. Adhesive removal test

The adhesive removal test was modified from that of Schallert et al. [16]. Briefly, the animal was placed in a novel environment (cage) and allowed to explore for 5 m. Then round adhesives (1.9 cm diameter, Avery) were placed on the pad of both forelimbs (ventral side of forelimb) and the animal was returned to the environment. Animals were given a total of 300 s to sense and/or remove the adhesives. The time to acknowledge the adhesive and the time to remove the adhesive were recorded for each forelimb. An acknowledgement of the adhesive included forelimb taxis or biting at the