Published in final edited form as:

Behav Brain Res. 2015 September 1; 290: 161–171. doi:10.1016/j.bbr.2015.05.001.

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

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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 hours 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 a significantly elevated frontal lobe brain water content 24 and 72 hours 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

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The authors have no conflicts of interest to report.

test, and the foot fault test since correlations with brain water content were observed at both 24 and 72 hours post-SBI.

Keywords

Neurobehavior; surgical brain injury; SBI; brain edema; experimental models; rats

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. 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 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.

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 hour light/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 (Figure 1A).

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 30–50 minutes.

Neurological Evaluations

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

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 evaluates spontaneous activity, body proprioception, vibrissae touch, limb symmetry, lateral turning, forelimb outstretching, and climbing. For spontaneous activity (SA), the animal was observed for 5 minutes 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). For climbing (CL), the animal was placed on a gripping surface (20×42 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).

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 seconds, then placed perpendicularly on the beam halfway between the platforms and given 40 seconds 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 seconds; 4, animal reached the platform between 25 and 40 seconds; 3, animal moved halfway to a platform and stayed on the beam for at least 25 seconds; 2, animal moved less than halfway on the beam and stayed on the beam for at least 25 seconds; 1, animal did not move and stayed on the beam for 40 seconds; and 0, animal fell off the beam in less than 25 seconds if animal moved along the beam or animal fell off the beam in less than 40 seconds if animal did not move.

Corner Turn Test—The corner turn test was conducted as previously described [15]. Animals were approached a 30 degree 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 seconds 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 $[(\text{left turns})/(\text{total turns}) \times 100]$.

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 $[(\text{left forelimb placements})/(\text{total number of trials}) \times 100]$.

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 minutes. 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 seconds 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 adhesive. When the adhesive fell off without the animal directly removing it, the trial was repeated.

Beam Balance Test—The beam balance test was performed to assess motor coordination, primarily balance and proprioception [17, 18]. A rectangular beam (3.2 cm wide, 122 cm long, 63.5 cm tall) was suspended between two tables (105 cm tall for the top of the beam). Animals were placed perpendicularly on the midpoint of the beam and allowed to traverse the beam for 180 seconds; videos were recorded for post-test analysis of the total distance traveled and the distance traveled until the animal first fell off the beam. For the

distance traveled until the animal's first fall, the distance traveled was used for animals which remained on the beam the full three minutes.

Foot Fault Test—The foot fault test was performed to assess fine motor skills and proprioception [19, 20]. A grid (90 cm × 25 cm, 2.5 cm grid spacing) was suspended 80 cm in the air between two platforms. Animals were placed on each platform for 30 seconds, then placed in the center of the grid. Videos were recorded of the animals as they traversed the grid for 120 seconds. Videos were post-test analyzed for the number of foot faults (i.e. each time a paw slipped through the grid) and the total number of steps per limb. The foot faults of each limb, front limbs (left and right forelimb), back limbs (left and right hindlimb), left limbs (left forelimb and left hindlimb), right limbs (right forelimb and right hindlimb), and total (all limbs) were expressed as a percentage of the respective number of steps. For example, the amount of left forelimb faults was computed via $[(\text{number of left forelimb faults})/(\text{total number of left forelimb steps}) \times 100]$, and the amount of left limb faults was computed via $[(\text{number of left forelimb faults}) + (\text{number of left hindlimb faults})/(\text{total number of left forelimb steps}) + (\text{total number of left hindlimb steps}) \times 100]$.

Evaluation of Brain Water Content

Post-neurological testing, brain edema was assessed by brain water content, measured via the wet weight/dry weight method [21]. Briefly, deeply anesthetized rats were decapitated at either 24 or 72 hours after surgery and the brains were quickly removed. Each hemisphere was separated into the frontal lobe, parietal lobe, cerebellum, and brain stem. Each section was weighed for the wet weight (APX-60, Denver Instrument, Bohemia, NY) then dried at 100°C for 48 hours. After drying, the samples were weighed for the dry weight. Brain water content (%) was calculated as $((\text{wet weight} - \text{dry weight})/(\text{wet weight})) \times 100$. Only right frontal lobe brain water content is presented in this study.

Statistical analysis

Brain water content (%) was presented as mean ± standard deviation (SD) and analyzed using one-way ANOVA with Tukey's *post-hoc* test (GraphPad Prism 6, La Jolla, CA, USA). Neuroscore (and its sub-tests) and beam walking score are presented as the mean score ± SD and analyzed using Kruskal-Wallis one-way ANOVA with Dunn's *post-hoc* test. The corner turn, forelimb placement, adhesive removal, beam balance, and foot fault tests are presented as mean ± SD and analyzed using one-way ANOVA with Holm-Sidak's *post-hoc* test. Correlation between functional deficits and brain edema was analyzed with the Spearman's rank correlation coefficient for Neuroscore and beam walking score. For the corner turn, forelimb placement, adhesive removal, beam balance, and foot fault tests, correlation between neurobehavior and brain edema was analyzed with the Pearson's product-moment correlation coefficient. The bestfit linear regressions are shown. A p-value of 0.1 was considered statistically significant.

Results

No mortality was observed in this study. The area of the resected tissue, analyzed using ImageJ was $6.06 \pm 0.993 \text{ mm}^2$ for the SBI group and $0.16 \pm 0.484 \text{ mm}^2$ for the sham group.

Brain Edema after Surgical Brain Injury

Partial frontal lobectomy resulted in elevated brain water content of the frontal lobe at 24 and 72 hours post-SBI compared to sham animals ($p < 0.05$ vs Sham for SBI (24 Hrs) and SBI (72 Hrs)). Frontal lobe brain water content was also significantly higher 72 hours after SBI compared to that of SBI animals sacrificed at 24 hours ($p < 0.05$) (Figure 1B).

Neurofunctional Deficits and Brain Edema Correlation

Prior to euthanasia at 24 or 72 hours after surgery, all animals were subjected to a series of neurofunctional assessments, consisting of the composite neuroscore, beam walking, corner turn test, forelimb placement test, adhesive removal test, beam balance test, and foot fault test, which all detected neurological deficits 24 hours post-SBI ($p < 0.05$ vs Sham). Similarly, all the tests, except adhesive removal test, yielded neurofunctional deficits 72 hours post-ictus ($p < 0.05$ vs Sham) (Figures 2–9).

Neuroscore—Significant functional deficits were observed in the composite neuroscore for animals subjected to SBI compared to sham animals at both 24 and 72 hours after injury ($p < 0.05$ vs Sham for SBI (24 Hrs) and SBI (72 Hrs)) (Figure 2A). For the individual subtests, only limb symmetry (LS) and forelimb outstretching (FO) had significantly different scores between the sham and SBI animals (LS: $p < 0.05$ vs Sham for SBI (24 Hrs) and SBI (72 Hrs); FO: $p < 0.05$ vs Sham for SBI (72 Hrs), $p > 0.1$ vs Sham for SBI (24 Hrs)) (Figure 2B). Although neuroscore was significantly lower for SBI animals than sham animals at both time points, the neuroscore did not significantly correlate with brain water content ($p > 0.01$ at 24 and 72 hours post-SBI) (Figures 2C and 2D).

Beam Walking Test—The ability for animals to walk on a beam was significantly impaired in SBI animals compared to that of sham animals ($p < 0.05$ vs Sham for SBI (24 Hrs) and SBI (72 Hrs)) (Figure 3A). At both time points, the beam walking score significantly correlated with brain water content ($p = 0.07$ for animals 24 hours post-ictus; $p < 0.01$ for animals 72 hours post-ictus) (Figures 3B and 3C).

Corner Turn Test—Animals subjected to SBI performed had significantly lower percentages of left turns than sham animals at both time points ($p < 0.05$ vs Sham for SBI (24 Hrs) and SBI (72 Hrs)) (Figure 4A). A significant correlation was observed between the percentage of left turns and the amount of brain edema at both time points ($p < 0.05$ for animals 24 hours post-ictus; $p = 0.1$ for animals 72 hours post-ictus) (Figures 4B and 4C).

Forelimb Placement Test—Similarly, the percentage of left forelimb placements were significantly worse in rats subjected to SBI compared to that of sham animals ($p < 0.05$ vs Sham for SBI (24 Hrs) and SBI (72 Hrs)) (Figure 5). The performance of SBI animals at 24 hours significantly correlated with brain water content ($p < 0.05$); however the performance of SBI animals at 72 hours did not ($p > 0.1$) (Figures 5B and 5C)

The percentage of right forelimb placements were not significantly different between any of the groups. Thus, the right forelimb placements did not correlate with brain water content ($p > 0.1$) (Supplementary Figure 1).

Adhesive Removal Test—Animals subjected to SBI took significantly longer to acknowledge and remove the adhesive from their left forelimb than sham animals at 24 hours after injury (Time to Acknowledge Left Limb Adhesive: $p < 0.05$ vs Sham for SBI (24 Hrs); Time to Remove Left Limb Adhesive: $p < 0.05$ vs Sham for SBI (24 Hrs)), but not at 72 hours (Time to Acknowledge Left Limb Adhesive: $p > 0.1$ vs Sham for SBI (72 Hrs); Time to Remove Left Limb Adhesive: $p > 0.1$ vs Sham for SBI (72 Hrs)) (Figures 6A and 6D). Both the time to acknowledge and remove the left limb adhesive significantly correlated with brain edema at both 24 hours (Time to Acknowledge Left Limb Adhesive: $p < 0.05$; Time to Remove Left Limb Adhesive: $p < 0.05$) and 72 hours post-SBI (Time to Acknowledge Left Limb Adhesive: $p = 0.1$; Time to Remove Left Limb Adhesive: $p = 0.08$) (Figures 6B, 6C, 6E, and 6F).

For the adhesive on the right forelimb, the time to acknowledge and remove the adhesive was not significantly different between any of the groups ($p > 0.1$), nor did either of them correlate with brain edema ($p > 0.1$) (Supplementary Figure 2). Additionally, the number of times the animals acknowledged the adhesive on the left forelimb was not significantly different between the sham and SBI animals at 24 hours ($p > 0.1$), yet there was significance between the SBI animals at 24 and 72 hours ($p < 0.05$) (Supplementary Figure 3A). The number of adhesive acknowledgements correlated with brain water content at 24 hours ($p = 0.08$), but not 72 hours post-SBI ($p > 0.1$) (Supplementary Figures 3B and 3C). Finally, the number of adhesive acknowledgements for the right forelimb adhesive was not significantly different between any of the groups, yet it seemed to correlate with brain water content at 24 hours post-ictus ($p < 0.05$) but not 72 hours post-SBI ($p > 0.1$) (Supplementary Figure 4).

Beam Balance Test—The ability of animals to balance and traverse on a beam was significantly worse for SBI animals at 24 and 72 hours after SBI compared to those of sham animals (Distance Traveled: $p < 0.05$ vs Sham for SBI (24 Hrs) and SBI (72 Hrs); Distance Traveled to First Fall: $p < 0.05$ vs Sham for SBI (24 Hrs) and SBI (72 Hrs)). SBI animals recovered some ability to traverse the beam 72 hours after injury compared to that of SBI animals at 24 hours post-SBI (Distance Traveled: $p < 0.05$ between SBI (24 Hrs) and SBI (72 Hrs); Distance Traveled to First Fall: $p > 0.1$ between SBI (24 Hrs) and SBI (72 Hrs)) (Figures 7A and 7D). The performance of SBI animals on the beam balance test significantly correlated with the magnitude of brain edema at 24 and 72 hours post-ictus for the distance traveled ($p < 0.01$ for animals 24 hours post-ictus; $p = 0.08$ for animals 72 hours post-ictus). The SBI animals' performance for the distance traveled to first fall only correlated with brain edema at 24 hours post-SBI ($p < 0.05$), but not at 72 hours ($p > 0.1$) (Figures 7B, 7C, 7E, and 7F).

The time to fall and number of falls when animals traversed the beam were significantly worse (fell faster, more falls) for animals 24 hours after SBI compared to sham animals ($p < 0.05$). However, both of these parameters were recovered by 27 hours after SBI ($p > 0.1$ vs Sham, $p < 0.05$ vs SBI (24 Hrs)) (Supplementary Figures 5A and 5D). However, neither of these parameters correlated with brain water content at 24 or 72 hours after injury ($p > 0.1$) (Supplementary Figures 5B, 5C, 5E, and 5F).

Foot Fault Test—The performance of animals after SBI was significantly worse, compared to that of sham animals, in the foot fault test for the percentages of front left, hind left, total left, and total foot faults at 24 and 72 hours after injury (Front Left Faults: $p < 0.05$ vs Sham for SBI (24 Hrs) and SBI (72 Hrs); Hind Left Faults: $p < 0.05$ between Sham and SBI (24 Hrs), $p < 0.1$ between Sham and SBI (72 Hrs); Left Limb Faults: $p < 0.05$ vs Sham for SBI (24 Hrs) and SBI (72 Hrs); Total Foot Faults: $p < 0.05$ between Sham and SBI (24 Hrs), $p < 0.1$ between Sham and SBI (72 Hrs)) (Figures 8A and 9A).

The percentage of front left limb faults correlated with brain water content at both 24 and 72 hours post-SBI ($p < 0.05$ for animals 24 and 72 hours after SBI); however the hind left limb faults did not ($p > 0.1$ for animals 24 and 72 hours after injury) (Figures 8B–E). The percentage of left limb faults correlated with brain edema at 24 hours ($p = 0.09$) but not 72 hours post-ictus ($p > 0.1$), while that of all limbs did not correlate with brain edema at either 24 or 72 hours post-ictus ($p > 0.1$ for animals at 24 and 72 hours after injury) (Figures 9B–E).

The foot faults for the right forelimb, right hindlimb, both front limbs, both hind limbs, and both right limbs were not significantly different between any of the groups ($p > 0.1$ between all group pairings), nor did they correlate with brain water content at either time point ($p > 0.1$ for all parameters at each time point) (Supplementary Figures 6 and 7).

Discussion

Surgical brain injury leads to three primary secondary injuries: post-operative hematoma, neuroinflammation, and brain edema. Following the primary insult, intra-operative bleeding leads to the formation of post-operative hematomas and cerebral edema, worsening post-SBI outcome [5, 8, 9, 22]. The SBI rodent model allows for the pathological mechanisms and new therapeutics to be investigated [5, 8, 9, 22]. While the SBI model most closely mimics injury found after tumor resection, the rodent SBI model has a similar pathophysiology as that observed in other neurosurgical procedures, including peri-operative hemorrhage, cerebral edema, and inflammation [5, 8, 9, 22].

Rats subjected to SBI presented with increased brain water contents 24 and 72 hours post-ictus compared to animals subjected to sham surgery. Additionally, SBI resulted in significant functional deficits 24 hours post-injury in all seven sensorimotor tests evaluated within, and agreed with the deficits observed by others [9, 22, 23]. These functional deficits were maintained for 72 hours after SBI in all neurobehavior tests except the adhesive removal test, also in agreement with previous studies [9, 23]. The apparent recovery of neurofunction in the adhesive removal test may be in part due to animals learning the task; all animals which were tested at 72 hours post-ictus were subjected to all seven functional exams 24 hours after SBI. This explanation may also be used to understand the slight recovery of animals' performances (at 72 hours after injury) on the beam balance test (distance traveled parameter).

Brain Edema Correlates with Functional Deficits 24 and 72 Hours after SBI

At 24 hours after SBI, the magnitude of brain edema, assessed using the brain water content changes, correlated with beam walking, left turns in the corner turn test, left forelimb

placements, time to acknowledge and remove an adhesive from the left forelimb, distance traveled and distance traveled until the animal fell in the beam balance test, and the amount of front left limb and left limb foot faults. For these parameters, as the severity of SBI increased (*i.e.* brain water content was higher), the performances of animals' worsened. This suggests that these parameters, and their respective behavior tests, can be used to assess the severity of SBI and amount of brain edema to aid in determining if a therapeutic improves functional outcome via a reduced brain water content.

At 72 hours post-ictus, brain edema correlated with beam walking, left turns in the corner turn test, the distance traveled on the beam balance test, and amount of front left limb foot faults. Similar to the data at 24 hours, as brain water content increased, animals' performance decreased.

The adhesive removal test (time to acknowledge and time to remove the left limb adhesive) showed a correlation with brain water content (for both parameters) at 72 hours. Intriguingly, rather than having a worse performance as brain water content increased, animals performed seemingly better. The correlation between the performance and brain water content may be caused by the discrepancy between the performances; animals either performed very well or very poorly. The animals likely performed better at the adhesive removal tasks because they performed the tasks previously (at 24 hours post-SBI). One indication that animals learned the task can be inferred from the time to acknowledge the adhesive. The time to acknowledge the adhesive is greatly smaller at 72 hours post-SBI compared to 24 hours post-ictus, whereas the time to remove the adhesive is indistinguishable. This suggests that although the animals may have either learned the task or have improved sensation, the motor deficits remained, preventing them from removing the adhesive. However, whether the animals learned the task or whether they actually recovered function remains to be studied.

Tests Lacking Brain Edema Correlation

The lack of correlation for the composite neuroscore (at 24 and 72 hours post-SBI) may be due to lack of sensitivity; while gross sensorimotor ability is evaluated in the neuroscore, fine sensorimotor ability is not. The observed functional deficits for SBI animals may be primarily due to the partial lobectomy since part of the sensorimotor cortex is removed [24], rather than increased brain edema.

Interestingly, the number of left forelimb placements correlated with brain edema 24 hours post-SBI, but not at 72 hours. This is likely caused by the observation that all SBI animals performed very poorly at this task 72 hours post-ictus, thus no correlation was found. At first glance, it may seem like this lack of correlation is due to the lobectomy, however, since the animals performance correlated with brain water content at 24 hours post-SBI, the increased magnitude of brain edema at 72 hours likely prevented any of the animals from performing well at this task.

For the beam balance test, a 72 hours after SBI, the distance traveled until the animal first fell does not correlate with brain water content. Similar to the forelimb placement test,

animals may be too severely injured to observe any correlation with performance and brain edema on this task at this time point.

Finally, the amount of left hindlimb and total foot faults do not correlate with brain edema at either 24 or 72 hours post-injury, nor does the amount of left limbs correlate with brain water content at 72 hours after injury. A reasonable explanation for the lack of correlation for the left hindlimb foot faults is that the partial lobectomy plays a greater role on the performance of the left hindlimb than brain water content (approximately 50% of the steps taken are faults). The lack of correlation for the total amount of foot faults may be due to worse left limb performance while there is no difference in right limb performance, thus the correlation between performance and brain edema is lost.

Lack of Brain Edema Correlation for Right-Sided Tasks

Since the SBI model utilizes a partial resection of the right frontal lobe, unilateral deficits are expected to dominate functional performance post-injury; animals should perform poorly at functional tasks when the ipsilateral side is required, but when the contralateral side is required, or when the contralateral side can compensate for ipsilateral deficits, little to no performance loss is expected. Therefore, for all tasks which utilized the contralateral side (right side and right limbs), little to no neurological deficits were observed (Supplementary Information).

Recommendations

Determining the correlation between cerebral edema and neurological function allows for studies which examine new treatments aimed at reducing edema to utilize the most appropriate tests. Based on the findings of this study, the recommended tests for this purpose are the beam walking, corner turn, beam balance (distance traveled and distance traveled to first fall), and foot fault (front left limbs only) tests. These tests had the best correlation with brain edema at 24 and 72 hours after SBI. While not specifically studied here, we recommend these tests also be utilized for other unilateral injuries of the brain.

If brain edema is not the specific target of a therapeutic in the rodent model of SBI, the neurobehavior tests which provide the most sensitivity to functional deficits are the beam walking, corner turn, forelimb placement, beam balance, and foot fault tests. We recommend these tests be used for functional assessment for any experimental SBI study regardless of the primary pathology studied.

Other Factors Contributing to Brain Edema and Neurological Deficits

The volume of tissue resected to induce SBI needs to be well-controlled as it may cause differences in brain edema and functional deficits. Thus it is critical for this parameter to be documented and similar for all animal groups. The correlation of resection volume with brain edema and consequent functional deficits remains to be examined in future studies.

Several other factors may also contribute to neurofunctional deficits after SBI, including surgery time and intra-operative hemorrhage. Variation in these factors may lead to varied amounts of iron and thrombin accumulation in the tissue as well as varied severity of

inflammation, which are known to induce brain edema [25]. Controlling the time of surgery and intra-operative hemorrhage will minimize the variability in brain edema between animals subjected to SBI, and should also be documented and reported.

Conclusion

Herein, the correlation between cerebral edema and neurofunctional deficits was examined after SBI in rats for seven common sensorimotor tests. This study can help determining the most relevant behavior tests to utilize for studying novel therapeutics which target brain edema. It is suggested that testing ipsilateral versus contralateral performance for exams which test fine sensorimotor ability, such as the beam walking, beam balance, and foot fault tests.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This work was supported by NIH R01 NS043338 and NS084921 grants (JHZ). The authors would like to thank Loic Adam, Guillaume Oudin, Jean-Sébastien Louis, and Corentin Walker for assistance with this work.

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Highlights

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

A. SBI Model **B. Brain Edema (24 and 72 Hrs post-SBI)** **Figure 1. Surgical Brain Injury Induces Brain Edema**

A. A representative SBI brain showing partial frontal lobectomy. **B.** Brain water content is significantly higher in animals subjected to SBI 24 and 72 hours post-ictus than sham animals. Brain water content is also higher 72 hours after injury compared to that 24 hours after SBI. Sham: n=8, SBI (24 Hrs): n=22, SBI (72 Hrs): n=11. * $p < 0.05$ vs Sham, # $p < 0.05$ vs SBI (24 Hrs).

A. Neuroscore (24 and 72 Hrs post-SBI)

B. Individual Test Scores (24 and 72 Hrs post-SBI)

C. Neuroscore and Brain Edema (24 Hrs post-SBI)

D. Beam Walking and Brain Edema (72 Hrs post-SBI)

Figure 2. Neuroscore

A. Neuroscore is significantly lower in SBI animals compared to sham animals. Sham: n=8, SBI (24 Hrs): n=38, SBI (72 Hrs): n=9. **B.** Individual sub-tests of the composite neuroscore. Only the limb symmetry (LS) and forelimb outstretching (FO) tests are significantly different between the groups. Individual tests: spontaneous activity (SA), body proprioception (BP), vibrissae touch (VT), limb symmetry (LS), lateral turning (LT), forelimb outstretching (FO), and climbing (CL). Sham: n=8, SBI (24 Hrs): n=38, SBI (72 Hrs): n=9. **C, D.** Neuroscore does not correlate with brain edema at either 24 or 72 hours after SBI. SBI (24 Hrs): n=23, SBI (72 Hrs): n=14. * $p < 0.05$ vs Sham.

Figure 3. Beam Walking

A. Beam walking is significantly reduced in animals subjected to SBI compared to sham animals. Sham: n=8, SBI (24 Hrs): n=23, SBI (72 Hrs): n=14. **B, C.** Beam walking correlates with brain edema at both 24 and 72 hours after SBI; the performances of animals' decreases as brain water content increases. SBI (24 Hrs): n=23, SBI (72 Hrs): n=14. * $p < 0.05$ vs Sham.

Figure 4. Corner Turn Test

A. The number of left turns is significantly reduced in animals after SBI. Sham: n=8, SBI (24 Hrs): n=32, SBI (72 Hrs): n=14. **B, C.** The number of left turns correlates with brain edema at 24 and 72 hours post-SBI; as brain water content increases, the performances of animals' decreases. SBI (24 Hrs): n=17, SBI (72 Hrs): n=14. * p<0.05 vs Sham.

Figure 5. Forelimb Placement Test

A. The number of left forelimb placements is significantly lower after SBI compared to sham surgery. Sham: n=6, SBI (24 Hrs): n=25, SBI (72 Hrs): n=14. **B, C.** The number of left forelimb placements correlates with brain edema at 24 hours post-ictus, but not at 72 hours post-SBI. At 24 hours after SBI, the performances of animals' decreases as brain water content. SBI (24 Hrs): n=17, SBI (72 Hrs): n=14. * p<0.05 vs Sham.

Figure 6. Adhesive Removal Test

A. The time to acknowledge the adhesive on the left limb is significantly higher for animals subjected to SBI and sacrificed 24 hours later, but not SBI animals at 72 hours after injury. Sham: $n=7$, SBI (24 Hrs): $n=36$, SBI (72 Hrs): $n=14$. **B, C.** Time to acknowledge the left limb adhesive correlates with brain edema at both time points. At 24 hours after SBI, animals' performances becomes worse at higher brain water contents. At 72 hours post-SBI, animals' performances seemly gets better as brain water content increases. SBI (24 Hrs): $n=23$, SBI (72 Hrs): $n=14$. **D.** The time to remove the left limb adhesive is significantly higher for animals subjected to SBI and sacrificed 24 hours after, and tends to be higher at 72 hours post-SBI. Sham: $n=7$, SBI (24 Hrs): $n=36$, SBI (72 Hrs): $n=14$. **E, F.** Time to remove the left limb adhesive correlates with brain edema at both time points. Similar to the time to acknowledge the adhesive, at 24 hours post-injury, the performances of animals gets worst as brain water content increases, but at 72 hours, higher brain water content correlates with better performance. SBI (24 Hrs): $n=23$, SBI (72 Hrs): $n=14$. * $p < 0.05$ vs Sham.

Figure 7. Beam Balance Test

A. Distance traveled on the beam is worse for animals subjected to SBI. Sham: n=6, SBI (24 Hrs): n=36, SBI (72 Hrs): n=14. **B, C.** Distance traveled correlates with brain edema at both time points; as brain water content increases, animals' performances gets worse. SBI (24 Hrs): n=23, SBI (72 Hrs): n=14. **D.** Distance traveled until the animal first falls off the beam is significantly less in SBI animals compared to sham animals. Sham: n=6, SBI (24 Hrs): n=36, SBI (72 Hrs): n=14. **E, F.** The distance traveled until first fall correlates with brain edema at 24 and 72 hours after injury; as brain water content increases, the performances of animals' worsens. SBI (24 Hrs): n=23, SBI (72 Hrs): n=14. * $p < 0.05$ vs Sham, # $p < 0.05$ vs SBI (24 Hrs).

A. Foot Fault Test (24 and 72 Hrs post-SBI)

B. Front Left Faults and Brain Edema (24 Hrs post-SBI)

C. Front Left Faults and Brain Edema (72 Hrs post-SBI)

D. Hind Left Faults and Brain Edema (24 Hrs post-SBI)

E. Hind Left Faults and Brain Edema (72 Hrs post-SBI)

Figure 8. Foot Fault Test

A. Foot faults for the front left and hind left limbs were significantly higher in SBI animals than that of sham animals. Sham: $n=6$, SBI (24 Hrs): $n=36$, SBI (72 Hrs): $n=12$. **B, C.** Front left foot faults correlates with brain edema 24 and 72 hours post-SBI; animals' performances worsens as brain water content increases. SBI (24 Hrs): $n=24$, SBI (72 Hrs): $n=14$. **D, E.** Hind left foot faults do not correlate with brain edema. SBI (24 Hrs): $n=24$, SBI (72 Hrs): $n=14$. * $p < 0.05$ vs Sham.

A. Foot Fault Test (24 and 72 Hrs post-SBI)

B. Left Limb Faults and Brain Edema (24 Hrs post-SBI)

C. Left Limb Faults and Brain Edema (72 Hrs post-SBI)

D. Total Foot Faults and Brain Edema (24 Hrs post-SBI)

E. Total Foot Faults and Brain Edema (72 Hrs post-SBI)

Figure 9. Foot Fault Test

A. Foot faults for the left limbs (front and hind left limbs) and all limbs (front left, front right, hind left, and hind right limbs) were significantly higher in SBI animals than that of sham animals. Sham: $n=6$, SBI (24 Hrs): $n=36$, SBI (72 Hrs): $n=12$. **B, C.** Left foot faults correlates with brain edema 24 post-ictus, but not 72 hours post-SBI. At 24 hours after injury, the performances of animals' worsens as brain water content increases. SBI (24 Hrs):

n=24, SBI (72 Hrs): n=14. **D, E.** Foot faults for all limbs does not correlate with brain edema at either time point. SBI (24 Hrs): n=24, SBI (72 Hrs): n=14. * $p < 0.05$ vs Sham.

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Table 1

Neuroscore Scoring Criteria for the Sub-Tests.

Sub-Test	Scoring			
	0	1	2	3
Spontaneous Activity (SA)	Animal was akinesitic	Animal moves slowly or minimally	Animal approached 1–2 walls	Animal approached at least 3 walls of the cage or raised on hindlimbs to explore the top of the cage
Body Proprioception (BP)	–	Animal had a unilateral response	Animal had either a weak bilateral response or weak left response and brisk right response	Animal had a brisk bilateral response
Vibrissae Touch (VT)	–	Animal had a unilateral response	Animal had either a weak bilateral response or weak left response and brisk right response	Animal had a brisk bilateral response
Limb Symmetry (LS)	Hemiparesis	Left forelimb or left hindlimb flexed	Asymmetric extension	All limbs were extended symmetrically
Lateral Turning (LT)	Animal had no turning at all on one side	Animal had unequal turning	Animal turned bilaterally less than 45 degrees on both sides	Animal turned bilaterally at least 45 degrees on both sides
Forelimb Outstretching (FO)	Animal had a paretic forelimb	Animal walked in circles	Animal walked asymmetrically or to one side	Animal briskly walked symmetrically on forepaws
Climbing (CL)	–	Animal failed to climb or circled instead of climbing	Animal climbed to the top and had a weak grip or animal climbed but had a strong grip	Animal climbed to the top and had a strong grip