A peer-reviewed version of this preprint was published in PeerJ on 3 January 2017.

View the peer-reviewed version (peerj.com/articles/2818), which is the preferred citable publication unless you specifically need to cite this preprint.

Kim HJ, Han SJ. 2017. A simple rat model of mild traumatic brain injury: a device to reproduce anatomical and neurological changes of mild traumatic brain injury. PeerJ 5:e2818 https://doi.org/10.7717/peerj.2818

A simple rat model of mild traumatic brain injury: device to reproduce anatomical and neurological changes of mild traumatic brain injury

Ho Jeong Kim, Soo Jeong Han

Mild traumatic brain injury typically involves temporary impairment of neurological function. Previous studies used the water pressure or rotational injury for designing the device to make a rat mild traumatic brain injury model. The objective of this study was to make a simple model of mild traumatic brain injury in rat. The device consisted of a free-fall impactor that was targeted onto the rat skull. The weight (175g) was freely dropped 30cm to rat's skull bregma. We installed a safety device made of acrylic panel. To confirm a mild traumatic brain injury in 36 Sprague–Dawley rats, we performed the brain magnetic resonance image(MRI) within 24 hours after injury. We evaluated behavior and chemical changes in rats before and after mild traumatic brain injury. The brain MRI did not show high or low signal intensity in 34 rats. The mobility on grid floor was decreased after mild traumatic brain injury. Absolute number of foot-fault and foot-fault ratio were decreased after mild traumatic brain. But the difference of ratio was lesser than absolute number of foot-fault. These results show that the device is capable of reproducing mild traumatic brain injury in rat. Our device can reduce the potential to cause brain hemorrhage and reflect the mechanism of real mild traumatic brain injury compared with existing methods and behaviors. This model can be useful in exploring physiology and management of mild traumatic brain injury.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.2037v1 | CC-BY 4.0 Open Access | rec: 10 May 2016, publ: 10 May 2016

Title: A Simple Rat Model of Mild Traumatic Brain Injury: Device to reproduce anatomical and neurological changes of mild traumatic brain injury

Authors: Ho Jeong Kim[1] , Soo Jeong Han [2]

[1] Department of Rehabilitation Medicine, Seonam hospital, Ewha Womans University Medical Center, Seoul, Republic of Korea

[2] Department of Rehabilitation Medicine, School of Medicine, Ewha Womans University, Seoul, Republic of Korea

Corresponding author :

Soo Jeong Han

Address: 1071, Anyangcheon-ro, Yangcheon-gu, Seoul 158-710, Republic of Korea

E-mail address: ocrystal@ewha.ac.kr

Introduction

A mild traumatic brain injury (MTBI) or concussion is referred to as a closed head injury, which may be defined as a temporary disturbance in brain function that occurs in a complicated pathophysiological process. In the United States, 3.8 million MTBIs occur during competitive sports and recreational activities. However, most of them have mild or no symptoms, and thus 50% of them go untreated (Collins et al. 1999). Actually, hospital-treated MTBIs are no more than 100 to 300/100,000 (Harmon et al. 2013). Neurological, cognitive and behavioral deficits, caused by MTBIs, are observed only for a short period of time. A headache, vomiting, cognitive slowing, fatigue, dizziness, depression, and problems with attention and memory can be one of its symptoms (d'Hemecourt. 2011; Ruff. 2011; Sherer et al. 2009). In the long run, it causes other post-concussive symptoms such as a learning disability, posttraumatic disorder, headache, dizziness, irritability, memory problem and otherwise (Holm et al. 2005). It has shown a high rate of incidence, but it is difficult to detect its symptoms. Accordingly, previous studies made rat models of MTBI to reveal the damaging mechanism and to find out the therapeutic method. The problem is that the rat model is made through a very complicated process of anesthesia and surgery, such as craniotomy followed by the insertion of a plastic injury tube or single impact therapy or hydraulic induction of concussion (Sakurai et al. 2012; Redell et al. 2013; Dixon et al. 1987; Dixon et al 1991). Recent study focused on the rat models of MTBI by considering the characteristics of MTBI, namely high-velocity and head acceleration (Kane et al. 2012). However, such a damaging mechanism, which delivered shock to their head and fell down them, could not induce MTBIs alone. In another study, shocks were delivered to the craniums of rats equipped with helmet disks, but it was also complicated to put the helmet disk (Xu et al. 2014).

In the case of a method suggested by Tang et al., it was comparatively simple and did not cause skull fractures (Tang et al. 1997). However, it caused brain edema that lasted about 48 hours. The purpose of this study was to develop a tool that can artificially cause MTBI in a safe and simple way and make the artificially-induced MTBI equal in the damaging mechanism to real MTBI. To confirm whether MTBI really occurred, a behavioral test was conducted on experimental rats. Moreover, the tool was inspected for safety with magnetic resonance imaging (MRI) scans and blood tests. The safety inspection was focused on critical injuries such as a skull fracture or cerebral hemorrhage and stress that affected homeostasis.

Materials and Methods

Animal groups

36 adult male Sprague-Dawley rats (200-250g, 7 weeks-old) were used. Animals were maintained on a 12-hours light/ 12-hours dark cycle and at constant temperature (21-24℃), and food and water were available ad libitum. All manipulation and experimental procedures on rats were approved by the local Ethics Committee (Ewha Medical Research Institute, No ESM 14-0252).

Mild traumatic brain injury procedure

This weight drop device model was modified from a protocol originally developed for mice as

described by Tang et al (Tang et al. 1997). Closed head MTBI was produced using a weight loss device. We fixed a rat on the wooden plate (25 x 30 cm^2) with Velcro, and 175g novel weight was dropped on the bregma of the rat. For decreasing risk of skull fracture, acryl plate was placed above the head of the rat. The drop height (from top to acryl plate) was 30cm, and the weight was gone through a polyvinyl chloride tube (inner diameter 11cm, height 30cm) to offer regular drop height. The plastic tube had small holes with regular intervals (2cm) to reduce air resistance (Figure 1).

Neurologic evaluation

Grid-walking and foot-fault test

Grid-walking and foot-fault test were performed just after the MTBI. The apparatus was consisted of an elevated 52 x 40 cm^2 metal grid with grid cell of 3 x 3 cm^2 (Barbosa et al. 2015). It was elevated 30cm above the floor, and the metal grid was made of stainless steel. The rats were placed in the center square of the apparatus, and they were free to explore for 1 minute. Behaviors in the grid were recorded with a video camera. For the 1 minute observation period, following parameters were quantified: (a) the total number of footsteps of hind limb, (b) the number of hind limb, (c) the foot fault ratio (the number of foot fault / the total number of footsteps) and (d) the latency (time to move after placing on metal grid). Foot fault tests were performed before and after 5 minutes of the MTBI.

Rota rod

Rota rod tests were performed before and after 10 minutes of Grid-walking and foot-fault test.

It was carried out by placing a Rota rod treadmill (Metal roller diameter 40mm, speed tachometer 15 rpm). The rod was divided into five equal segments with 9cm intervals. A rat was placed on the roller, and the time the rat stayed on it was measured (Tiwari et al. 2015).

Magnetic resonance imaging and blood sampling

All in vivo brain magnetic image (MRI) was performed 24 hour after MTBI. The MRI confirmed presence of the skull fracture, brain hemorrhage and diffuse axonal injury. MRI scans were carried out with a four-element phased-array animal-dedicated 5-cm inner diameter surface coil (Chenguang Medical Technology, Co., Ltd, Shanghai, China). T2 weighted images were taken using a standard spin echo sequence (TE 22ms; TR 650ms; slice thickness 3.00mm; matrix scan 512; FOV 100.00mm). In addition, subclavian veins were punctured to obtain blood samples to measure electrolytes, plasma glucose, and plasma calcium before as well as 5 and 20 minutes after MTBI.

Statistical Analysis

Comparisons of measurements between before and after MTBI were performed using paired-t test. Statistical analysis was performed using SPSS ver. 20.0 (IBM SPSS, Armonk, NY, USA) and p-values under 0.05 were considered significant.

Results

A total of 36 rats were applied to concussion model. The MR images of 34 rats were appeared normal. Only two rats had small amount subarachnoid hemorrhage. There was no intracerebral hemorrhage, skull fracture, diffuse axonal injury or death (Table 1, Figure 2). It takes to materialize MTBI for about 1-2 minutes. 34 rats, appeared normal in MRI, showed a significant decrease of the total number of foot step in foot fault test (p-value < 0.001). The mean of number of the foot step was 40.12 ± 10.96 before MTBI, and 40.12 ± 10.96 after MTBI.

Decrease aspects of action were shown on metal grid for 1 minute. Foot fault step before MTBI was 2.21 ± 1.61 per minute which had error rate of 0.07 ± 0.09 in the total foot step. After MTBI, however, the foot fault step was decreased to 0.59 ± 0.74 per minute (p-value < 0.001) with the decreased error rate of 0.04 ± 0.06 on the total foot step (p-value = 0.01).

For Rota rod evaluation, the rats maintained rolling with balance for 10.00 ±11.21 seconds before MTBI. They were able to keep rolling for 13.97 ± 16.09 seconds after MTBI, thus there was no significant difference of Rota rod evaluation between before and after MTBI (Table 2). Latency before MTBI was 0.94 ± 1.41, but it was prolonged to 5.26 ± 11.39 seconds after MTBI with statistical significance (p-value = 0.02).

There is no significant difference between before and after MTBI in blood test. The sodium level was shown no significant difference between before and after MTBI (before 138.34 ± 3.33 mmol/L; after 138.45 ± 1.88 mmol/L), as well as the potassium level (before 5.38 ± 0.17 mmol/L; after 4.98 ± 0.11 mmol/L). Decrease of serum glucose level was detected from 210.77 ± 49.33 mg/dL before MTBI to 196.00 ± 42.04 mg/dL after MTBI, but this result was not statistically significant. Calcium level change was also detected from 1.17 ± 0.27 to 1.21 ± 0.17 mmol/L with no statistical significance as well (Table 3).

Discussion

This study is aimed to make a new MTBI model that is equal in the damaging mechanism to MTBI. The tool was made with the modification of the method suggested by Tang et al. that used a weight to deliver a shock to the heads of rats (Tang et al. 1997). Because the physical impact, suggested by Tang et al., was judged to be too big, we reduce the drop height and put an acryl sheet between the rat head and the weight that it could absorb the shock from the weight. The study of Tang et al. focused on presence of just skull fracture, but did not describe serious outcome such as brain hemorrhage. In this regard, side effect of our method was compared to study of Kane et al. that have been used in many studies. In the study of Kane et al., a 95-gram weight was dropped at a height of 1 meter onto foil on which a rat stayed. The weight shock made the rat fall onto the sponge cushion that was 10 centimeters below the foil, which induced high-velocity and head acceleration. The method has widely been used to make a tool that causes MTBI in rats (Kane et al. 2012). In the study of Kane et al., skull fractures, intracranial bleeding, respiratory arrests and seizures occurred in 10% of rats, and the mortality rate reached 5%, but it has so far been recognized as safe. On the other hand, in this study, subarachnoid hemorrhage occurred just in 5.5%, 2 out of 36 cases. In addition, any mortality did not occur. These results imply that this study can be safer than others, and in particular, it could conduct an experiment within 1 or 2 minutes and does not need an incision and surgery, unlike previous studies (Sakurai et al. 2012; Redell et al. 2013; Dixon et al. 1987; Dixon et al 1991).

The grid-walking and foot-fault test is known to test sensorimotor coordination in neurological diseases such as a cerebral infarction, cortex injury, Parkinson’s disease that may be affected by

motor ability (Zhang et al. 2002; Barth et al. 1990; Shanina et al. 2006; Chao et al. 2012). This study well showed the characteristics of rats with MTBI, by applying the grid-walking and foot fault test. In the foot fault test, rats on the metal grid showed behavioral delay after getting MTBI. In several cases, any movements were not observed for about one minute. A delay in latency results from temporary unconsciousness that occurs after MTBI, or may have a possibility of being caused by post-concussive symptoms such as a headache, dizziness and irritability. To sum up, rats became slow in movement on the metal grid, which considerably reduced the number of their steps in addition to latency. Moreover, many rats laid almost moveless. It seems to be an aspect resulting from alterations in plasticity and activation and from hypometabolism as in the study of Shrey et al (Shrey et al. 2011). Reductions in foot-fault steps and foot-fault error rate does not result from improvement in sensorimotor coordination after MTBI but are more likely to be caused by reduction in real movements.

The Rota rod test is to examine balance impairments. The test detected the problems of balance and postural equilibriums subsequent to the occurrence of MTBI (Guskiewicz. 2011). In this study, there was no statistical significant difference regarding Rota rod rolling duration before and after MTBI. It may be because the Rota rod test was conducted after the grid-walking and foot-fault test. The rats of this study recovered from MTBIs faster and were less injured than those of other studies. The one thing that should not be overlooked is that the rats were in a fidget after getting MTBI and tended to have difficulty rolling, but measurements could not be conducted.

Pathophysiological studies on MTBI have been carried out with various specimens including cerebrospinal fluids, brain cells and serums, wherewith the studies have clarified the efflux of potassium ions into extracellular fluid, and the influx of sodium ions into the interior of cells in

the acute phase. The cellular depolarization temporarily causes the disruption of cell homeostasis, and increases the level of intracellular calcium ions (Clifton et al 1981; Domínguez DC, Raparla M. 2014; Giza and Hovda. 2014). Then, post-traumatic catecholamine is released and glycolysis occurs (Clifton et al. 1981; Shrey et al. 2011). In this study, the levels of serum electrolyte, glucose and calcium were measured 20 minutes after the induction of MTBI. As in previous studies, calcium accumulation occurred but was not a statistically significant change. Given that it was measured with serum, it is presumed that calcium accumulation was marginal. According to the report of Giza et al., glycolysis reaches its peak 6 minutes after the induction of MTBI, and hyperglycolysis ends 20 minutes later, and for 24 hours afterwards, the cerebral glucose metabolism slows down (Giza and Hovda. 2001). In this study, a blood test was conducted 20 minutes after the induction of MTBI, and so hyperglycolysis could not be observed. In addition, though a change occurs in the electrical charge of the membrane, the imbalance of electrolytes in real blood was not observed due to homeostasis.

This study has several limitations. First, hyperglycolysis could not be analyzed because the blood test was conducted 20 minutes after the induction of MTBI because of behavioral test. Second, 36 experimental rats is considered a small number for the study. It is possible, of course, to generalize the rat models of MTBI with the use of parametric statics, yet a larger number of experimental rats might be helpful to raise the validity and reliability of this study.

This study made it possible to realize the rat model of MTBI with safety and simplicity. The traumatic brain injuries, induced in this study, were much milder than other studies. It is expected to be a great help to study pathophysiology or progress of patients who do not visit hospitals on the excuse of mild symptoms and comprises 50% of patients with MTBI. Furthermore, it may be applied to studies on repetitive MTBI that are underway.

Ackowledgement; The authors wish to thank Ms. Mina Hong for support conducting the trials.

References

Barbosa EH, Vallim JH, Lachat JJ, de Castro VL. Assessments of Motor Abnormalities on the Grid-Walking and Foot-Fault Tests From Undernutrition in Wistar Rats. J Mot Behav 29: 1-8, 2015.

Barth TM, Jones TA, Schallert T. Functional subdivisions of the rat somatic sensorimotor cortex. Behavioral Brain Research 39: 73–95, 1990.

Chao OY, Pum ME, Li JS, Huston JP. The grid-walking test: Assessment of sensorimotor deficits after moderate or severe dopamine depletion by 6-hydroxydopamine lesions in the dorsal striatum and medial forebrain bundle. Neuroscience 202: 318–325, 2012.

Clifton GL, Ziegler MG, Grossman RG. Circulating catecholamines and sympathetic activity after head injury. Neurosurgery 8(1): 10-14, 1981.

Collins MW, Grindel SH, Lovell MR, Dede DE, Moser DJ, Phalin BR, Nogle S, Wasik M, Cordry D, Daugherty KM, Sears SF, Nicolette G, Indelicato P, McKeag DB. Relationship between concussion and neuropsychological performance in college football players. JAMA 282: 964–970, 1999.

d'Hemecourt P. Subacute symptoms of sports-related concussion: outpatient management and return to play. Clin Sports Med 30: 63–72, 2011.

Dixon CE, Clifton GL, Lighthall JW, Yaghmai AA, Hayes RL. A controlled cortical impact model of traumatic brain injury in the rat. J Neurosci Methods 39: 253–262, 1991.

Dixon CE, Lyeth BG, Povlishock JT, Findling RL, Hamm RJ, Marmarou A, Young HF, Hayes RL. A fluid percussion model of experimental brain injury in the rat. J Neurosurg 67: 110–119, 1987

Domínguez DC, Raparla M. Neurometabolic aspects of sports-related concussion. Semin Speech Lang 35(3): 159-165, 2014

Giza CC, Hovda DA. The Neurometabolic Cascade of Concussion. J Athl Train 36(3): 228-235, 2001.

Giza CC, Hovda DA. The new neurometabolic cascade of concussion. Neurosurgery 75(4): 24-33, 2014.

Guskiewicz KM. Balance assessment in the management of sport-related concussion. Clin Sports Med 30: 89–102, 2011.

Harmon KG, Drezner JA, Gammons M, Guskiewicz KM, Halstead M, Herring SA, Kutcher JS, Pana A, Putukian M, Roberts WO. American Medical Society for Sports Medicine Position Statement: concussion in sport. Br J Sports Med 47: 15-26, 2013.

Holm L, Cassidy JD, Carroll LJ, Borg J. Summary of The WHO Collaborating Centre For Neurotrauma Task Force On Mild Traumatic Brain Injury. J Rehabil Med 37: 137-141, 2005.

Kane MJ, Angoa-Pérez M, Briggs DI, Viano DC, Kreipke CW, Kuhn DM. A mouse model of human repetitive mild traumatic brain injury. J Neurosci Methods 203(1): 41-49, 2012.

Redell JB, Moore AN, Grill RJ, Johnson D, Zhao J, Liu Y, Dash PK. Analysis of functional pathways altered after mild traumatic brain injury. J Neurotrauma 30(9): 752-764, 2013.

Ruff RM. Mild traumatic brain injury and neural recovery: rethinking the debate. NeuroRehabilitation 28: 167–180, 2011.

Sakurai A, Atkins CM, Alonso OF, Bramlett HM, Dietrich WD. Mild Hyperthermia Worsens the Neuropathological Damage Associated with Mild Traumatic Brain Injury in Rats. J Neurotrauma 29(2): 313–321, 2012.

Shanina EV, Schallert T, Witte OW, Redecker C. Behavioral recovery from unilateral photothrombotic infarcts of the forelimb sensorimotor cortex in rats: Role of the contralateral cortex. Neuroscience 139: 1495–1506, 2006.

Sherer M, Yablon SA, Nakase-Richardson R. Patterns of recovery of posttraumatic confusional state in neurorehabilitation admissions after traumatic brain injury. Arch Phys Med Rehabil 90: 1749–1754, 2009.

Shrey DW, Griesbach GS, Giza CC. The pathophysiology of concussions in youth. Phys Med Rehabil Clin N Am 22(4): 577-602, 2011.

Tang YP, Noda Y, Hasegawa T, Nabeshima T. A concussive like brain injury model in mice (I): impairment in learning and memory. J Neurotrauma 14: 851– 862, 1997.

Tiwari HS, Tripathi AK, Mishra DP, Kalita J, Misra UK. A study of ER stress in rat model of cerebral venous sinus thrombosis. Neurosci Lett 589: 121-125, 2015.

Xu L, Nguyen JV, Lehar M, Menon A, Rha E, Arena J, Ryu J, Marsh-Armstrong N, Marmarou CR, Koliatsos VE. Repetitive mild traumatic brain injury with impact acceleration in the mouse: Multifocal axonopathy, neuroinflammation, and

neurodegeneration in the visual system. Exp Neurol 275: 436-449, 2016.

Zhang L, Schallert T, Zhang ZG, Jiang Q, Arniego P, Li Q, Chopp M. A test for detecting long-term sensorimotor dysfunction in the mouse after focal cerebral ischemia. Journal of Neuroscience Methods 117: 207–214, 2002.

Figure 1: Device for mild traumatic brain injury using a rat as subject. The components of the devices are a vertical guide tube for the dropped weight and an acryl panel to absorb impact.

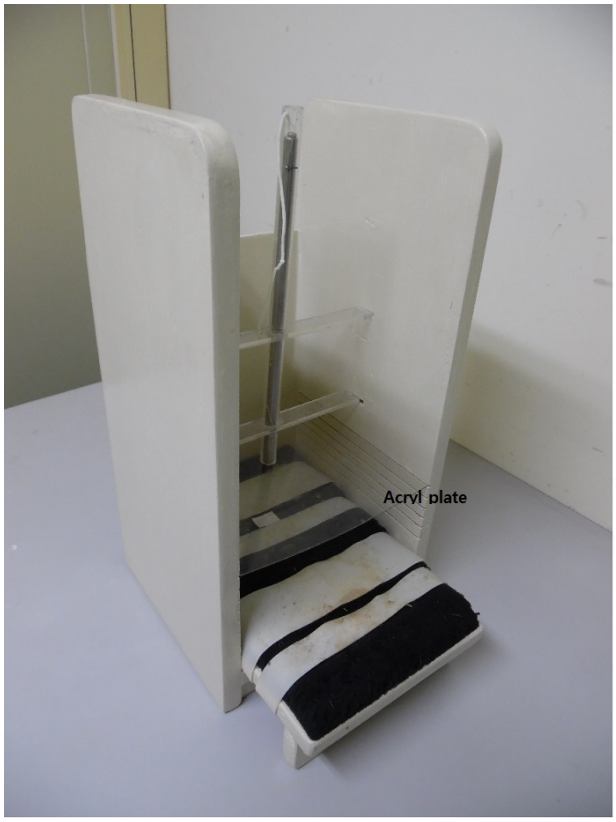


Figure 2: Finding of magnetic resonance imaging after mild traumatic brain injury. There were no significant cerebral hemorrhage, intracranial hemorrhage, and diffuse axonal injury.

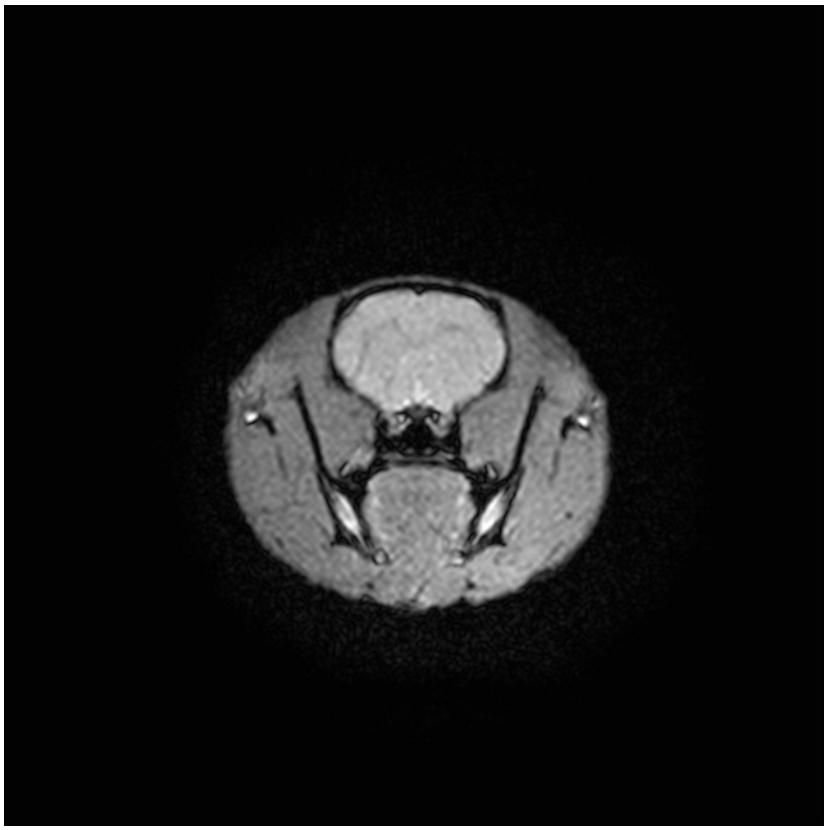
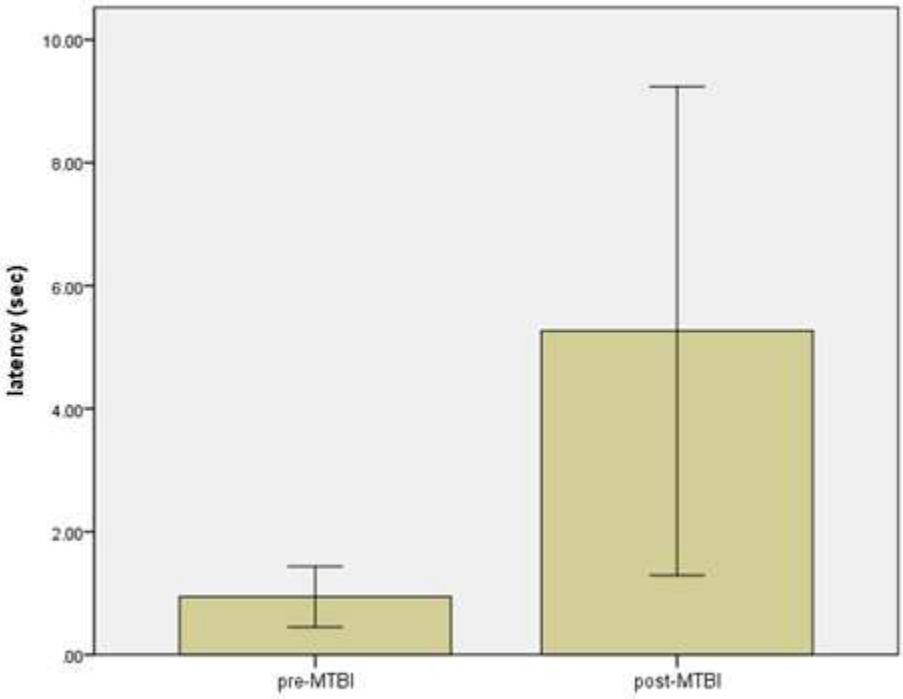


Figure 3: Movement latency on metal grid was delayed after mild traumatic brain injury ($p<0.05$). The latency of pre-MTBI was 0.94 ± 1.41 second, but the latency of post-MTBI was 5.26 ± 11.39 second.



MTBI, mild traumatic brain injury

Sec, second

306 Table 1: Anatomical change after injury.

307

308

	Number
Total number of enrolled rat	36
Mild traumatic brain injury	34
Subarachnoid hemorrhage	2
Intraventricular hemorrhage	0
Intracerebral hemorrhage	0
Skull fracture	0
Diffuse axonal injury	0

309

310

311

312

313

314

315

316

Table 2: Comparisons of rota rod and foot fault test parameters between before and after mild traumatic brain injury.

	Total foot step (number/min)	Foot fault step (number/min)	Foot fault ratio	Time on rota rod (sec)
Before MTBI	40.12 ± 10.96	2.21 ± 1.61	0.07 ± 0.09	10.00 ± 11.21
After MTBI	17.50 ± 14.50	0.59 ± 0.74	0.04 ± 0.06	13.97 ± 16.09
p-value	<0.001	<0.001	0.01	0.21
95% CI	17.45 to 27.79	1.05 to 2.18	0.01 to 0.06	-10.22 to 2.28

Values are mean ± standard deviation

MTBI, mild traumatic brain injury; Sec, second; Min, minute

Table 3: Comparisons of serum parameters between before and after mild traumatic brain injury.

	Sodium (mmol/L)	Potassium (mmol/L)	Glucose (mg/dL)	Calcium (mmol/L)
Before MTBI	138.34 ± 3.33	5.38 ± 0.17	210.77 ± 49.33	1.17 ± 0.27
After MTBI	138.45 ± 1.88	4.98 ± 0.11	196.00 ±42.04	1.21 ± 0.17
p-value	0.87	0.65	0.06	0.51
95% CI	-1.33 to 1.28	-0.03 to 0.83	18.82 to 92.79	-0.16 to 0.08

Values are mean ± standard deviation

MTBI, mild traumatic brain injury; Sec, second; Min, minute

342

343

344

345

346

347

348

349