Journal of Neuroscience Methods 233 (2014) 18–27

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Basic Neuroscience

# A novel traumatic brain injury model for induction of mild brain injury in rats

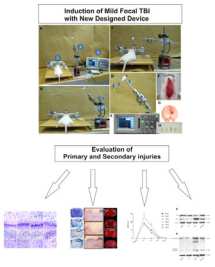
Tahereh Ghadiri[a,b], Mohammad Sharifzadeh[a,c,**], Fariba khodagholi[d], Sayed Mostafa Modarres Mousavi[b], Gholamreza Hassanzadeh[a], Mohammad-Reza Zarrindast[a], Ali Gorji[b,e,f,*]

[a] School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
[b] Shefa Neuroscience Research Center, Tehran, Iran
[c] Faculty of Pharmacy, Department of Toxicology, Tehran University of Medical Sciences, Tehran, Iran
[d] Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[e] Institute of Physiology I, Department of Neurosurgery, Epilepsy Research Center, Münster University, Germany
[f] Institute of Physiology I, Department of Neurology, Epilepsy Research Center, Münster University, Germany

## HIGHLIGHTS

- Mild TBI constitute approximately 80% of all traumatic brain injuries in humans.
- Improved animal models that mimic all aspects mild TBI in humans are needed.
- A novel stereotaxic coupled weight drop device was designed.
- The new device induced both primary and secondary damages at trauma site.
- This new model of TBI is suitable for evaluation of mild TBI.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 3 April 2014
Received in revised form 26 May 2014
Accepted 28 May 2014
Available online 4 June 2014

*Keywords:*
Brain injury
Weight drop
Dark neurons
Head trauma
CNS

## ABSTRACT

*Background:* Due to the marked heterogeneity of human traumatic brain injury (TBI), none of the available animal model can reproduce the entire spectrum of TBI, especially mild focal TBI. This study was designed to develop a modified TBI weight drop model for induction of focal mild cerebral injury.
*New method:* A stereotaxic coupled weight drop device was designed. Principle arm of device carries up to 500 g weights which their force was conveyed to animal skull through a thin nail like metal tip. To determine the optimal configuration of the device to induce mild TBI, six different trials were designed. The optimal configuration of the instrument was used for evaluation of behavioral, histopathological and molecular changes of mild TBI.
*Results:* Neurologic and motor coordination deficits observed sharply within 24 h post injury period. Histological studies revealed a remarkable increase in the number of dark neurons in trauma site. TBI increased the expression of apoptotic proteins, Bax, BCl2 and cleaved caspase-3 in the hippocampus.
*Comparison with existing methods:* Our designed TBI device is capable to produce variable severity of TBI from mild to severe. The main advantage of the new TBI model is induction of mild local unilateral brain

* Corresponding author at: Epilepsy Research Center, Universität Münster, Robert-Koch-Straße 27a, D-48149 Münster, Germany. Tel.: +49 251 8355564; fax: +49 251 8355551.
** Corresponding author at: Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
*E-mail address:* gorjial@uni-muenster.de (A. Gorji).

http://dx.doi.org/10.1016/j.jneumeth.2014.05.035

injury instead of traumatization of the whole brain. This model does not require craniotomy for induction of brain injury.
*Conclusion:* This novel animal TBI model mimics human mild focal brain injury. This model is suitable for evaluation of pathophysiology as well as screening of new therapies for mild TBI.


## 1. Introduction

Numerous events, such as falls, sports, motor accidents and military injuries, can lead to traumatic brain injury (TBI). TBI is a leading cause of mortality and morbidity in the population below 50 years with a peak incidence in young adults (Bruns and Hauser, 2003; Marklund and Hillered, 2011). More than 1.5 million people worldwide die due to TBI each year. The number of TBI victims globally raised sharply in the last few decades (Tagliaferri et al., 2006). TBI can cause long-term physical disability, neurobehavioral deficits and disrupt quality of life (Masel and DeWitt, 2010). Mild TBI constitute approximately 80% of all traumatic brain injuries in humans (Jennett, 1998). The deficits produced by mild TBI are frequently more subtle, less often recognized, and more contentiously debated than are those resulting from severe TBI (Dikmen et al., 2001).

In spite of the developments in the prevention, diagnosis and management of TBI, there is still no adequate treatment available to ameliorate its disabling effects. In view of the heterogeneous nature of the clinical situation in TBI, numerous animal models of such injury have been created to study the underlying mechanisms of human TBI. The purpose of experimental models of TBI is to replicate certain pathological components or phases of clinical trauma in experimental animals aiming to address pathophysiological mechanisms and develop preclinical therapeutics (Marklund et al., 2006; Marmarou and Povlishock, 2006). The major techniques of experimental TBI models are including the controlled cortical impact, weight-drop, impact acceleration and fluid percussion models (Morales et al., 2005; Cernak, 2005). A variety of morphological, cellular, molecular, bioelectrical and functional changes of human TBI, including alterations in ionic homeostasis, generation of free radicals, eliciting neuroinflammatory responses, releases of excitatory amino acids, initiation of negative DC shifts (spreading depolarization) and changes in neurotransmitter systems have been characterized by various experimental TBI models (Finnie and Blumbergs, 2002). However, there is no single animal model of TBI that can reliably mimic all aspects of human TBI, especially mild focal TBI (Morales et al., 2005; Cernak, 2005; Finnie and Blumbergs, 2002). Improved animal models that reflect the relevant processes in mild TBI in humans, and in which new novel biomarkers might be identified and evaluated, are needed (Zetterberg et al., 2013). Traditional weight drop TBI models, such as impact acceleration model, have some disadvantages and limitations. Due to lack of enough control on forces induced by a weight drop, these models frequently destroy the majority part of the ipsilateral cortices and lead to central respiratory depression. Different weight-drop models usually require craniotomy or have a high probability of skull fracture and rebound injury. The results obtained by these models clearly not comparable with the extent of brain injuries observed in many of survivors of human TBI, especially those suffered from mild-to-moderate TBI. It is suggested that if TBI could be induced without craniotomy or skull fracture, it will be more ideal for studying human TBI (Morales et al., 2005). Cortical compact injury model allows for better control over mechanical factors, such as velocity of impact and depth of resulting deformation but is not able to accurately mimic bioelecterical and functional aspects of human TBI (Morales et al., 2005; Cernak, 2005). In order to fulfill the needs for a reliable model of focal TBI and cost effective device, the present study introduces a new method of focal brain injury via a novel weight drop TBI model.

## 2. Materials and methods

### 2.1. Apparatus arrangement

The overall arrangement of the new TBI device and its accessory components are shown in Fig. 1. The apparatus consists of:

1. Weights with various sizes and desired mass (40, 80, 100 and 200 g). The delivered force can be changed by assembling the different weights on to dropping arm (Fig. 1A–D).
2. Dropping arm which is relied on a metal rod in free end of a magnetic stand at the basement of apparatus. This part carries desired number of weights in longitudinal fashion. The maximum weight it can tolerate is 600 g (Fig. 1B).
3. Basement of device which is made from heavy metal disk ($5 \times 5$ cm) with magnetic property. Another metal rod (25 cm length) is projected from the central part of the basement. This part and basement are fixed part of the device. The angle between the metal rod and the dropping arm can be adjusted (30–120°) and provides diverse dropping heights. In this meet point, electrical energy converts to driving force of dropping arm falling, by pressing the button of trigger handle (Fig. 1A–C).
4. Multiple size tips (4 tips, various diameters in the encountering side, max area 10 $mm^2$), which can screw to a hammer like metal rod (7 cm length) connected to free end of dropping arm with a 90° angle (Fig. 1D–H).
5. Two connectors; one for supplying electricity source and have a trigger button, another one for connecting to oscilloscope (Fig. 1A).
6. A stereotaxic surgery device (Fig. 1A).

It should be mentioned that the height which weight launches from, varies depending on the angle between the principle arm and an assumptive horizontal line. In our method weight is not launched directly into skull and the force conveys through vertically dropping of hammer like metal rod due to release of principle arm by trigger button. Launching of the dropping arm is controlled via pressing button of trigger connector. The force of weights transmits to the skull through tip of the hammer like metal rod. The force of dropping weight on the head of animal was measured by a force sensor (FSG15N1A, Honeywell, Germany; sensitivity 0.24 mV/g, measurement range 0–1500 g) located on the tip of hammer like metal rod. The signal from the sensor is amplified by a homemade amplifier (1 V/kg) and the pressure pulse of the injury was recorded on a storage oscilloscope triggered by the fall of the dropping arm. The amplitude of the pulse was used to determine the intensity of the weight drop force.

Two experimental protocols were conducted. The first one was aimed to determine appropriate weight, angle and height to induce a mild-to-moderate focal TBI. Using histopathological and molecular investigations, the second series of experiments were designed to confirm induction of brain injury induced by the new TBI device.