Cognitive deficits, behavioral changes and gene expression profiling in a mouse model of repeated mild traumatic brain injury

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Abstract

Repeated mild traumatic brain injury (rmTBI) has been thought to result in cumulative damage to cells of the brain, but the molecular mechanisms are not known. We investigated the effect of rmTBI on cognition, behavior and hippocampal gene expression using microarray analysis. Mice applied with rmTBI for 25 successive days and tested at 1 week and 4 weeks after the injury respectively, showed transient neurological deficit, impaired weekly body weight growth rate (BWGR), changed behaviors in elevated plus maze and deficit in spatial memory in the Morris water maze compared with sham injury mice. Microarray analysis suggested several rmTBI-induced expression differences in intracellular signaling, apoptosis and cell cycle, angiogenesis, cellular architecture, inflammation, oxidative stress, metabolism and transcriptional regulation, and neuronal plasticity-related genes. This study highlights some of the potential mechanisms that may play an important role in the development of rmTBI-induced functional deficits. Further studies at different time points and in additional subregions of the brain are of interest in the search for molecular mechanisms behind rmTBI-induced neuronal pathogenesis after the injury.

Preface

Animal experiment protocols were approved by the University of British Columbia Animal Care and Use committee.

The number of the certificate of ethics approval is A06-0007.

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Chapter 1: Introduction

1.1 Traumatic brain injury (TBI)

1.1.1 Definition

Traumatic brain injury (TBI) is defined as structural injury and/or physiological disruption of brain function resulting from an external mechanical force such as the head being struck by or striking of an object, acceleration and/or deceleration movement, penetrated by a foreign object, impact generated from a blast or explosion, and so on. Not all individuals suffering from a traumatic force will sustain a TBI, but anyone who has exposed to such an external force with immediate manifestation of at least one of the following clinical signs could be considered to have had a TBI (TheManagementofConcussion/mTBIWorkingGroup, 2009).

- a period of loss of or a decreased level of consciousness (LOC)
- a state of inability to remember events that occur right before or after the injury (post-traumatic amnesia [PTA])
- a state of confusion in which the person is disoriented or with slowed thinking or any alteration in mental state (alteration of consciousness/mental state [AOC])
- neurological deficits (weakness, loss of balance, amongst other) that may or may not persist
- intracranial lesion

1.1.2 Classification of TBI

TBI is most often classified by one of three main systems: physical mechanism,

injury severity and pathological features (Saatman et al., 2008).

Depending on the mode of trauma (the causative forces), TBI could be classified as closed or open injury. Closed injury is also called blunt injury which typically occurs when the head is moving impulsively or when the head is brought to a sudden rapid stop without being struck (noncontact or “inertial” loading). Another type of closed TBI is caused by impact loading, which occurs when the head strikes an object or vice versa. And this type of injury is usually associated with both contact and inertial forces (JE., 1998).

While the open injury occurs when sharp objects pierce the scalp, skull and usually the meninges and underlying brain tissue (contact or “impact” loading). In this classification, the type and severity of injury may be predicable from the magnitude and direction of each type or combination of loading forces. For example, impact loading will mostly results in focal injuries, such as skull fracture, brain contusion, and epidural hematoma, whereas inertial loading generally produces more diffuse injuries such as concussion, subdural hematoma and diffuse axonal injury, and so on.

Based on severity, TBI could be classified into mild, moderate, and severe categories. The 15-point Glasgow Coma Scale (GCS) (Teasdale and Jennett, 1974) is the most commonly used system for classifying TBI severity in adults, because of its high inter-observer reliability and generally good prognostic capabilities (Narayan et al., 2002). The scale grades the patient’s level of consciousness based on verbal, motor, and eye-opening reactions to stimulus (Parikh et al., 2007). Although there is no consistent

definition about the severity, it is generally agreed that a TBI with a GCS of 13 or above is mild, 9–12 is moderate, and 8 or below is severe (Jennett, 1998; Tim Anderson et al., 2006; Valadka, 2004).

Nevertheless, the GCS grading system has limited ability to predict outcomes. Because of this, other classification systems such as a current model developed by the Department of Defense and Department of Veterans Affairs use all the three criteria of GCS after resuscitation, duration of loss of consciousness (LOC), alteration of mental state (AOC) and post-traumatic amnesia (PTA) to determine the injury severity (Table 1).

The terms concussion and mild TBI (mTBI) may be used interchangeably (Kelly and Rosenberg, 1997; TheManagementofConcussion/mTBIWorkingGroup, 2009). For centuries, the term concussion has been commonly used in sports medicine and it is the most common type of traumatic brain injury. Although there is no consistent definition of concussion or mTBI, it has been commonly accepted that concussion is a complex pathophysiological process affecting the brain, resulting from traumatic biomechanical forces and involving temporary impairment of neurological function that quickly recovers by itself and with no gross structural changes observed in neuroimaging (Petchprapai and Winkelman, 2007).

Based on pathological features of the injury, TBI could be classified as focal or diffuse injuries. In fact, it is common to see both focal and diffuse damages in the same patient after TBI. Focal injuries are commonly associated with direct mechanical forces such as the head being struck by or striking an object. Another common event in which

the focal injuries often occur is penetrating head injury, with the dura mater, the outer layer of the meninges, is breached. In focal injuries, symptoms are typically related to the damaged area of the brain. Diffuse injuries, unlike focal injuries which are confined to specific areas, distributed in a more general manner and they often result from acceleration/deceleration injuries, hypoxia, meningitis, and damage to blood vessels. Diffuse injury manifests with little apparent damage when detected by neuroimaging, but much of the damage is microscopic.

1.1.3 Epidemiology

TBI is one of the leading health and socioeconomic problems in the world. The cost of hospitalization and treatments has raised great concerns by the health care system. In the recent years more than 350,000 people have been admitted annually due to TBI in USA alone (Kraus and McArthur, 1996). The social and economical cost is so large that it has reached 38 billion dollars per year (Faul M et al., 2010).

The number of hospitalization and deaths resulting from TBI is a significant concern in the United States. Each year, more than 52,000 people died from severe traumatic brain injury and it continues to be a leading cause of death and morbidity in North America (Cassidy et al., 2004; Thurman et al., 1999). 300,000 patients are also hospitalized due to moderate trauma and more than 1.3 million treated for concussions or other forms of mild TBI (Adekoya et al., 2002).

mTBI has raised great interest as 80-85% of the TBI are considered to be mTBI (Graham DI et al., 2002). However, mTBI is much more difficult to determine due to

factors such as varying definitions and possible under-reporting and under-diagnosing. So this problem is often difficult for the clinicians to manage and can cause frustration for patients. mTBI incidence estimates vary, but are generally around 100–600 per 100,000 people annually (D'Ambrosio and Perucca, 2004; Holm et al., 2005; Park et al., 2008). mTBI incidences could be affected by age, gender, race and several other factors (Hofman et al., 2001).

1.2 Repeated mild traumatic brain injury (rmTBI)

1.2.1 Epidemiology of rmTBI

There is an emerging hypothesis in the field of TBI research is that rmTBI may have a cumulative effect on the brain and will ultimately lead to clinical signs such as cognitive deficits. Actually, rmTBI does happen a lot in some specific areas especially in sports (Kelly and Rosenberg, 1997). In contact sports which include blows on the head such as boxing and some martial arts, recurrent mTBI is common and inevitable. In collision sports, like soccer, hockey, and American football, some researchers also recorded higher frequency of repeated concussion (JE., 1998). In soccer, heading the ball is an accepted way to play the game and it has become a mechanism for potential brain injury (Matser et al., 2001). Studies showed that soccer players who head the ball more often would have more brain concussion and worse results on multiple neuropsychological tests (Matser et al., 1999; Matser et al., 1998). In American football, players who had multiple concussions perform worse on neuropsychological tests than those only had a single or no concussion (Collins et al., 1999). rmTBI is also a common

consequence of boxing since victory in boxing is to render the opponent unconscious with a concussion. Actually, rmTBI can result from collisions or falls in all forms of athletic activity. Besides, child abuse victims and spousal abuse also contribute to the number of rmTBI (Roberts et al., 1990; Shannon et al., 1998). Although the cumulative effect of multiple mTBI is poorly understood, a series of long-term problems are believed to be associated with multiple concussions, even when incidents are separated in time by months or years (Harmon, 1999). Persons with the third and subsequent concussions may have worse symptoms in neuropsychological tests (Maroon et al., 2000).

1.2.2 Symptoms

Awareness of the detrimental effects of repetitive concussions and subconcussions has existed for decades. The early example can be traced back to 1928, when a forensic pathologist first described the tremors, abnormal behavior, progressive cognitive decline, and speech problems as dementia pugilistica (DP) which developed progressively over the course of a career in boxing (Cantu, 2007). Recently, the aftermath of multiple concussions has been observed in numerous professional athletes who have had their careers prematurely ended due to these injuries. In a study initiated with the intent of demonstrating the cumulative effects of concussion in junior hockey players, it has been shown that young hockey players with three or more concussions differed significantly in post-concussion symptoms and cognitive event-related potentials than players with zero concussion (Gaetz et al., 2000). In a large study performed in US university football athletes, players with a history of two or more concussions had lowered baseline

neuropsychological performance and reduced cognitive performance in two tests designed to measure information processing speed than athletes with no previous concussions (Collins et al., 1999). In a study evaluating the frequency of concussion in Swedish ice hockey players, it was reported that multiple concussions could cause the manifestation of permanent brain injuries in neuropsychological test without abnormal EEG and CT (Tegner and Lorentzon, 1996). In American football, it has been reported that the risk of having a concussion is 4 to 6 times greater if the player had already sustained a concussion compared with those who has no history of concussion (Kelly and Rosenberg, 1997). In professional soccer players, it has been found that the increase in the number of concussion correlated with poorer performance in memory, as well as planning and visual perception (Matser et al., 1998). The same group of authors also found the dose-response relation between the number of headers and cognitive functions among soccer players. That is more headers in one professional season, poorer results on focused attention and visual / verbal memory tests (Matser et al., 2001). This result was in agreement with a MRI study which demonstrated that compared with American football players, soccer players showed more MRI abnormalities while the number of concussions between the two group were the same. And they thought the cumulative effect of headers may explain the differences they found (Autti et al., 1997). Some researchers thought repeated subtle injury may have a cumulative effect just like other medical problems caused by repeated low-level activity, such as lung cancer from cigarette smoking, skin cancer from sun exposure, and so on (Babbs, 2000).

1.2.3 Experimental models

We have gained some information about symptoms and pathology of rmTBI by studying it directly from people who sustained such kind of injury. However, it is very hard to study the cellular and molecular mechanism except in very rare cases when athletes die as a result of the insult. So it is necessary to turn to experimental models of rmTBI to gain such knowledge.

In experimental studies of repetitive TBI, the repetitive injuries should be defined as an initial external mechanical force to the head followed by another mechanical insult. It is different from the so called secondary injury which has a delayed onset and progression over hours to days and months after the initial trauma and includes brain swelling, raised intracranial pressure (ICP), and hypoxic/ischemic brain damage and so on.

Some experiments have used large animals such as non human primate, pigs or dogs to establish TBI models. Rodent models on the other hand have been more commonly employed, because of their advantages like small size, easy of performing surgery and behavior tests, not expensive cost, large number available, remain the first choice of most TBI studies.

1.2.3.1 Experimental models of rmTBI in vivo

It is very vital to use animal models to understand the underlying mechanism of the highly complicated pathophysiology following TBI. Since rmTBI generally occurs at a mild level, experimental models mimicking it should be minimally invasive and do not

require a craniotomy.

1.2.3.1.1 Weight drop model

In the weight drop model of TBI, the gravitational forces of a free falling, guided weight is used to produce head injury. In the early weight drop model, the head of the unanesthetized mouse was restricted to a styrane foam pillow by holding its pinnae and was then given impact by dropping a bakelite rod, 20 g in weight, 10 mm in diameter, over the parietal region from the height of 30 cm. Then they measured the duration to regaining of righting reflex and spontaneous movement. The mice without brain contusion (macroscopic damage or bleeding like petechiae or hematoma of the brain tissue) in the following autopsy were considered to have concussion like brain injury (Manaka and Sano, 1978). In another similar rat model, the rat was lightly anesthetized and the scalp was cut and separated. And then a desired distance was chosen according to the rat's weight and a blow was then delivered to the exposed skull. The velocity of the falling weight can be measured by a piezoelectric accelerometer. This model produced brain edema and pathological changes similar to those found in clinical TBI patients (Shapira et al., 1988). In a model attempting to reproduce concussive-like (mild) TBI without brain contusion and focal lesion, the transient neurobehavioral suppression, short duration of brain edema and long-lasting deficits of learning and memory were observed to be similar to those of human concussive brain injury (Tang et al., 1997). In one study using repetitive concussive brain injury regimen developed in the mouse weight drop model, spatial learning deficit was observed (DeFord et al., 2002). In another weight drop

impact model, multiple episodes of mTBI were observed to result in transient， reversible loss of consciousness and cognitive impairment in Morris water maze (Creeley et al., 2004).

1.2.3.1.2 Controlled cortical impact (CCI) model

The CCI model utilizes a rigid impactor to deliver mechanical energy from pressurized air to the exposed dura. This model was first characterized in ferrets (Lighthall, 1988) and then adapted to rats (Dixon et al., 1991) and mice (Smith et al., 1995). Behavioral testings have revealed that transient deficits of motor function such as loss of forelimb and hindlimb reflex (Nakamura et al., 1999), rotarod deficits (Shear et al., 2004) and decreased strength post-injury (Raghupathi et al., 1998). Cognitive deficits have also been revealed by the Morris water maze in CCI mice, even one year after the initial injury (Fox et al., 1999; Shear et al., 2004). CCI model predominantly causes a focal brain injury mimicking a spectrum of contusion injuries in clinical patients. After CCI in the rodent, it has been reported that low injury levels produced no significant macroscopic or microscopic change, but higher injury levels produced cortical contusion, intraparenchymal hemorrhage and blood-brain barrier disruption (Dixon et al., 1991). It has also been observed that cortical cell loss, hippocampus cell loss and ventricular expansion occurred post CCI injury in the rat and these damages can be seen in the contralateral hemisphere at higher magnitudes of injury (Goodman et al., 1994). The severity of the injury was determined mainly by cortical compression and impact velocity (Dixon et al., 1991), which gives the advantage of this model to be easy to control

deformation parameters with pneumatically driven devices and thus the ability to control the severity of injury. Since 2001, some studies have used a mild CCI model which is thought to most closely mimic the type of insults that athletes may receive in order to investigate the behavioral and pathological changes associated. In one such study, the researchers used a silicone impactor to distribute the impact energy over a large area of the exposed skull and avoided skull fracture. They found that in the group of animals subjected to a second injury 24 h later, there was an increase of cortical BBB breakdown, axonal injury and impairment in neuroscore rotarod and rotating pole (Laurer et al., 2001). In a study that examined the effect of time intervals between different episodes and the number of traumatic insults, the researchers induced TBI in mice at 3,5,7 days apart, respectively. The results showed that mice receiving the second impact within a 3-day to 5-day interval exhibited significant deficits in both the rotarod test and Morris water maze test, as well as wide-spread axonal injury, which were not evident in sham animals or those injured only once (Longhi et al., 2005).

1.2.3.2 Experimental models of repetitive mTBI in vitro

The in vitro models offered cellular and molecular insights of this pathology and complement the findings from humans and from in vivo experimentation.

The stretch-induced injury of in vitro approach originated from Ellis et.al (Ellis et al., 1995), which utilized astrocytes grown in culture. Briefly, rat (1 to 2 days old) cortical astrocytes were cultured into tissue culture well with a collagen-coated 25-mm diameter silastic membrane as its bottom surface. When the cells were confluency and then a rapid

positive pressure of known amplitude (psi) and duration (msec) was produced by a cell injury controller. The cells growing on the membrane were thus stretched following the deformation of the membrane, which was proportional to the amplitude and duration of the air pressure pulse. Some researchers have utilized this in vitro stretch-induced injury model to conduct a series of studies investigating the effects of repeated trauma on hippocampal cells. One example was the study utilizing a mild level of stretch injury to produce some measurable damage to the cells when administered a single time. But when this injury was repeated either 1hr or 24hrs later, the cells showed a loss of neurons and released a neuron specific enolase (NSE), which was not observed in single injured cells. Also, the effect of the time interval between different episodes of insults on the damage extent was revealed. That is cells which received the second insult 1h after the first one released more S-100B protein (a clinical marker for nervous system damage) than cells that received the second insult at 24 hrs later (Slemmer et al., 2002). In another study, the researchers applied a low level of stretch to the cultured cells which did not cause any observable damage to the culture when administered a single time or even repeated 1h apart. But when the time interval was reduced to 2 minutes, increased neuronal loss and NSE release were observed which exhibited cumulative damage of repeated mild injury (Slemmer and Weber, 2005).

1.3 Hippocampus and memory

Hippocampus is an important component of the brain which plays important roles in long-term memory and spatial navigation. Patients with hippocampus damage (in

accident or in disease like Alzheimer's, hypoxia, amongst others) exhibit memory problems and disorientation which appear to be the most common symptoms. In rodents, the hippocampus has been studied extensively as part of a brain system responsible for spatial learning and memory, behavioral inhibition and so on. The rodents with hippocampus damage tend to be hyperactive and have difficulty learning to inhibit responses that they have previously been taught. The latter gave rise to the theory of the role of the hippocampus in anxiety (Gray and McNaughton, 2000). Several studies have reported impaired hippocampal LTP after TBI in vivo (Albensi, 2001; Weber, 2004) and cumulative damage to hippocampal cells in vitro (Slemmer et al., 2002). Since some studies also correlated the ability of neurons to undergo changes in synaptic plasticity, such as long-term potentiation (LTP) with learning and memory (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999), one area of current and future researches could focus on pathophysiology of synaptic plasticity and its underlying mechanism after injury (such as LTP), as well as correlated hippocampus-mediated behavioral changes.

1.4 Objective and specific aims

1.4.1 Objective and hypothesis

In the clinic, increasing evidence suggests that individuals with repeated concussions (mTBI) are more vulnerable to having other concussions, require longer periods of time to regain their baseline functioning, have longer lasting cognitive and emotional dysfunctions such as memory deficits, depression and so on and higher vulnerability to neurodegenerative disorders like Alzheimer's disease. Similar to human mTBI cases,

injured animals have demonstrated deficits in memory and learning too. The more recent in vitro experiments using cells grown in culture have demonstrated that repeated mild injury can cause cumulative damage to hippocampal cells, which complemented the findings from humans and from in vivo experimentation. Although we have gained knowledge about the pathophysiological changes after rmTBI, the underlying mechanisms remain unknown. Therefore, the main objective of this study is to examine the hypothesis that rmTBI will trigger differential gene expressions which result in neurological dysfunctions, cognitive deficits and behavioral changes following the injury.

1.4.2 Specific aims

This thesis tries to find the underlying molecular mechanisms associated with neurological dysfunctions, cognitive deficits and behavioral changes following rmTBI in a mouse model through the following specific aims:

1. To evaluate the acute neurological responses, weekly body weight growth rate and motor function following rmTBI.

2. To examine behavioral changes and cognitive deficit following rmTBI by doing elevated plus maze and Morris water maze.

3. To detect underlying gene expression changes in hippocampus after rmTBI by doing Illumina whole genome gene expression profiling.

Chapter 2: Materials and methods

2.1 Animals

20 male and 20 female C57BL/6 mice of 10-week old at the beginning of the experiment were used. They were housed in group of 5/cage in our animal facility under standard conditions (22±1℃) with a 12:12 hr light- dark cycle (lights on at 6:00 am). Water and food were continuously available. All mice were housed in Animal Research Center for 2 weeks for adapting to the laboratory environment prior to the initiation of treatment. The animal experimental protocol was approved by The University of British Columbia Animal Care and Use committee.

In both male and female groups, 10 mice were designated as control and 10 as TBI. All the TBI mice received the concussive-like head injury once a day for 5 successive weeks. After the 25 repeated head injuries, all the mice were divided into two batches with one batch tested at 1 week after the injury and another one tested at 4 weeks after the injury. The time line of the whole experiment is shown in Fig.1.

2.2 Weight drop device

As shown in Figure 2, the weight drop device consists of a hollow Plexiglas tube 2cm in diameter and 40 cm long, a cylindrical-shaped acrylic stick (20g) and a foam platform. The tube is kept vertical to the surface of the mouse head and guides the freely falling acrylic stick onto the head. The diameter of the upper part of the stick is 1.8cm, which allows little lateralization of the stick to hit the head. The end of the stick is flat, round and 1cm in diameter which will hit the top of the mouse head encompassing the

area over the frontal and parietal bones.

2.3 Production of rmTBI

To produce the mild head injury, mouse was anesthetized by 2.5% isoflurane (a widely used inhalation anesthetic drug). Disappearance of extremity reflex when it was stimulated by a needle on the toe was considered to be suitable for injury. Then the mouse was quickly placed in a prone position on the platform so that the mouse head was closely underneath the lower opening of the Plexiglas tube. Next, the stick was released from 25cm height. After consciousness (indicated by return of righting reflex and mobility) was regained, the mouse was put back to its home cage. The control mouse was anesthetised and placed on the platform in the same fashion as the injured ones, but without any injury.

2.4 Acute neurological evaluation

Acute neurological evaluation was performed every day right after the injury was delivered by recording the time of loss of consciousness (LOC). LOC was evaluated by the duration of the loss of righting reflex (LORR). LORR was measured as the time interval between loss of the righting reflex (the mouse did not right itself after being placed on its back) and regain of the righting reflex (the ability of the animal to re-right itself 3 times consecutively after being placed on its back) (Ferko, 1986).

Convulsion was also recorded throughout the acute evaluation period. Briefly, the mouse was placed in a clear Perspex observation box right after the injury was delivered. While recording for the time of LORR, the number and intensity of convulsion were also

recorded. The duration of each convulsion was graded as short (1 to 10 s), medium (11 to 30 s), or long (≥ 31 s). According to the Charles River Laboratories grading system (Silverstone et al., 2008), the intensity of each convulsion was classified into mild, moderate, and severe as defined by: mild = head and tail slightly extended and little jerking; moderate = head and tail fully extended and some jerking; severe = head and tail fully extended and strong jerking.

Visible foreleg paralysis was recorded if the forelegs were paralyzed visibly following the injury for more than 1 hour.

2.5 Weekly body weight growth rate (weekly BWGR)

Body weight was measured at the beginning of the injury and then at the same time of every week throughout the experiment. The weekly body weight growth rate was calculated as following: Weekly body weight growth rate＝【（body weight—body weight of the previous week）/ body weight of the previous week】×100

2.6 Motor function evaluation

In order to measure grip strength and balance, the endurance of wire hanging test was measured by placing the mouse on top of a wire mesh (1×1 cm grid) which was taped around the edge and suspended 50cm above a soft bedding material. The mesh was gently shaken so that the mouse griped the wire and then it was turned upside down and the amount of time spent holding on to the mesh with all the four legs was recorded, up to a maximum of 60 seconds.

2.7 Elevated plus maze

The EPM was made of grey plastic and consisted of two open arms without walls (35 x 5 cm) and two enclosed arms with walls (35 x 5 x 15 cm). The arms extended from a central platform (5 x 5 cm). The maze was located 40 cm above the floor by four metal legs and it was directly under the incandescent light in the ceiling so that there were similar levels of illumination on both open and closed arms. The mice were carried to the testing room 1 hour before testing and stayed in their home cages until experiment was performed. At the beginning of the experiment, each mouse was placed at the central platform facing the same open arm. Navigation was monitored for a 5-min interval using ANY-maze, a video-tracking system from Stoelting Co., Wood Dale, IL, USA.

Locomotion of individual animals was calculated as total distance travelled during the 5 min test. The number of entries into the open arms and the time spent on the open arms were recorded automatically by the software. An arm entry was counted when all the four paws were on the arm. At the end of each test, the maze was cleaned with 75% ethanol and dried with paper towels.

2.8 Morris water maze

A 1.5-m-diam, water-filled, cylindrical tank was used to perform Morris water maze. Extra-maze visual cues with different colors and shapes for orientation were permanently placed on the four walls around the tank. The temperature of the water in the tank was kept constant at 22±1°C. A 10-cm-diam platform was placed in a certain quadrant of the tank. The procedure consisted of 1 day of visible platform tests and 4 days of hidden

platform tests, plus a probe trial 24 h after the last hidden platform test. In the visible platform test, the platform was lifted 0.5cm above the water surface in the southeast, northeast, northwest, southwest quadrant and the center of the pool respectively in each trial. There were 5 contiguous trials, with an intertrial interval of 1 hour. Mice were placed next to and facing the wall of the tank successively in north (N), east (E), south (S), and west (W) positions. In each trial the mouse was allowed to swim until it found the platform, or until 60 seconds had elapsed. In the latter case, the mouse was guided to the platform where it remained for 20 seconds before being returned to the cage. In the hidden platform tests, the platform was placed 0.5cm below the water surface in the southeast quadrant and mice were trained for 5 trials per day from all the N, E, S, W positions with an intertrial interval of 1 hour. The probe trial was conducted by removing the platform and placing the mouse next to and facing the N side. The time spent in the previously platform quadrant (southeast quadrant) was measured in a single 60 seconds trial. Tracking of animal movement was achieved with ANY-maze video-tracking system.

2.9 RNA preparation

The brains of 6 mice (3 mice from control and TBI group respectively) in the first batch which were tested at 1 week after TBI were used to extract RNA from hippocampi. Briefly, on the next day after all the behavioral tests were done, the mice were decapitated and the brains were extracted and hippocampi were separated and stored immediately at -80℃. On the following day, total RNA was isolated from hippocampi using the TRI Reagent (Sigma-Aldrich, Inc., St. Louis, MO) according to manufacturer's

protocol. The final RNA from each mouse had the A_{260}/A_{280} ratio≥1.7 and the samples were stored at -80℃ for future use.

2.10 Illumina whole genome gene expression assay

From an input total RNA of 500 ng, synthesis, amplification, and labeling of complementary RNA (cRNA) with Cy3 dye were performed according to the manufacturer's protocols using the Illumina TotalPrep RNA Amplification Kit (Applied Biosystems Inc., Foster City, CA, Catalog # : AMIL1791). The labeled cRNA samples were then assessed for quality by measuring $A_{260}/A_{280.}$ All of them fall in the range of 1.7 to 2.1. The Illumina's MouseWG-6 v2.0 Expression BeadChip containing more than 45,200 well annotated RefSeq transcripts allowed 6 samples to be interrogated in parallel on a single Bead chip. Labeled and amplified cRNAs (1.5μg/array) were hybridized to Illumina's Sentrix® Mouse-6 Expression Bead chips at 58°C for 16 h according to Illumina® Whole-Genome Gene Expression with IntelliHyb Seal System Manual. The array was washed and stained with 1 μg/ml cyanine3-streptavidin then scanned using an Illumina BeadStation 500 G - BeadArray Reader (Illumina, Inc., San Diego, CA). Reference, hybridization control, stringency and negative control genes were checked for proper chip detection. The results were extracted with the Illumina's BeadStudio software with quantile normalization and background subtraction. The Illumina custom error model with multiple testing correction (Benjamini & Hochberg false discovery rate) was applied to dataset to identify genes differentially expressed following injuries (filtered by Illumina's "detection p-value＜0.05" and Diff. p-value＜

0.05).

2.11 Functional classification

Except several genes with unknown function, the other altered genes with fold change ≥1.2 were assigned to one of 11 functional classes following extensive literature searches. The assignment of functional annotations was based on information in publicly available sources including Gene Ontology and PubMed. It is worth noticing that a given gene may have multiple functions and that a variety of assignment systems can be constructed. In our study, each gene was assigned to only one functional class.

2.12 Data analysis and statistics

Data in the acute neurological evaluation (eg. duration of loss of righting reflex) were analyzed by repeated measures factor ANOVA and two-way ANOVA. The other data from weekly body weight growth rate, elevated plus maze, motor function evaluation and Morris water maze were analyzed by two-way ANOVA and two-tailed Student's t-test. For all comparisons, analyses were performed in the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 18 and statistical significance was determined to be $P < 0.05$.

Data were organized into graphical form using GraphPad Prism 4 software (GraphPad Software, La Jolla, CA). All data are presented as means ±SE.

Chapter 3: Results

3.1 Acute neurological deficits

Acute neurological responses were evaluated by recording and comparing the duration of loss of righting reflex, the incidence of convulsion and visible foreleg paralysis. According to the classic definition, mTBI (concussion) was defined to have 30 minutes or fewer of loss of consciousness (LOC), 24 hours or fewer of post-traumatic amnesia (PTA), and a Glasgow Coma Scale (GCS) score of at least 13. Previous research studying single concussive-like brain injury in a similar mouse model found that the duration of loss of righting reflex generally correlated with the impact heights, and at the injury height of 25cm, the mean duration was approximately 1.6 min (Tang et al., 1997). In the current study, after single time injury, the male TBI mice had an average duration of loss of righting reflex of 103.7±10.7 sec (mean±S.D.) and the female TBI mice had an average of 104±8.6 sec. While the control male and female mice had the average of 12.3±3.0 and 12.1±3.1 sec respectively (Fig. 3). In order to test if duration of loss of righting reflex had any tendency to change with the number of rmTBI, Mauchly's test of sphericity was first applied to validate repeated measures factor ANOVA in both male and female mice. Since the P value of the Mauchly's test was <0.01, Greenhouse-Geisser corrections for violations of sphericity was applied. In the result of test of within-subjects effects, the P value of D (day of injury) and P value of D * Group (interaction between D and group) were >0.05, which meant that duration of loss of righting reflex did not change with the number of injuries in all the four groups (TBI-male, control-male,

TBI-female and control-female). At last, two-way ANOVA with Bonferroni correction was employed to detect if there was any difference between the four groups on each day of injury. The result showed that every day after the injury, there was a significant difference of duration of loss of righting reflex between TBI and their control in both sex, but there was no difference between male and female TBI or between male and female control. In brief, with the number of injuries increasing, the difference between TBI and control still exist in both male and female. But there was no significant increase or decrease of the duration within the male and female TBI mice respectively (Fig. 4). Besides, there was no death, skull fracture and convulsion happened following everyday's injury and there was one case of visible foreleg paralysis occurred in male and female TBI respectively.

3.2 Weekly body weight growth rate (weekly BWGR)

The body weights of all the mice were measured weekly starting at the beginning of the injury till the end of the behavioral tests. Since weekly BWGR was different between male and female control mice (two-way ANOVA, data not shown), the analysis was done by analyzing data of male and female separately. In the batch of mice tested at 1 week after rmTBI, as shown in Fig. 5A, male TBI mice had lower weekly BWGR than their control at the 1^{st} ($P<0.01$) and 4^{th} week ($P<0.01$) during the injury was applied daily. At the end of 5^{th} week, when the injury ended, the TBI mice recovered to the same extent as the control. But at the 7^{th} week, when encountered the behavioral test (Morris water maze), the weekly BWGR of the TBI mice become higher than that of the control ($P<$

0.01). And again, right after the stimulus of behavioral test, the TBI mice recovered to the same extent. Analysis of the female mice tested at 1 week after rmTBI gave a similar result as shown in Fig. 5B. Fig 5C and 5D show the weekly BWGR of the batch of mice tested at 4 weeks after rmTBI over a period of 11 weeks. Like the previous batch of mice, both the male and female TBI mice had transient lower weekly BWGR ($P < 0.01$) during the five weeks of injury application and these deficits recovered to the same level as the control by the end of the injury. But when encountered environmental stimuli like behavioral tests, the TBI mice would possess a higher weekly BWGR than the control ($P < 0.05$). This change of weekly BWGR will be gone after the finish of the stimuli.

3.3 Motor function evaluation

In case that the motor dysfunction following TBI could contribute to the observed behavioral changes, the wire hanging test was utilized to examine muscular strength and motor neuron integrity. As shown in Fig. 6, at both 1 week and 4 weeks after rmTBI, there was no difference of time hanging on the wire inversely between TBI and their control in both male and female mice. All of them can hang on the wire for at least 60 sec, indicating that there was no motor dysfunction at those two time points after rmTBI.

3.4 Elevated plus maze

In the elevated plus maze, the total distance travelled in the maze during the 5 min testing was utilized as an indicator of locomotion. In comparison with those of the control mice, the total distance travelled in the maze of the TBI mice were not significantly different, in both male and female mice and at both 1 week and 4 weeks

after the injury (P＞0.05), as shown in Fig. 7A, 7C, 7E and 7G. At 1 week after the injury, two-way ANOVA showed that there was a significant interaction of treatment by sex (P=0.048). So the data were split by sex and student's t test was used to compare between TBI and control in the two genders, respectively. Results showed that at 1 week after the injury, the female TBI mice spent much more time in the open arms than their control (P=0.017) (Fig. 7D) which indicated an increased impulsivity in the female mice after rmTBI. While there was no significant differences observed in the male mice (P=0.83) (Fig. 7B). At 4 weeks after the injury, the interaction of treatment by sex was still significant (P=0.022). Analysis of male and female data indicated that the difference of time spent in the open arms in the female group disappeared (P=0.42) (Fig. 7H), and the male TBI mice showed an increased time in the open arms (P=0.03) (Fig. 7F).

3.5 Morris water maze

Since memory impairment is among the top complaints of patients after concussive brain injury and patients with repeated mild TBI, it is important to examine whether rmTBI will lead to memory deficit in our mouse model. Morris water maze was used to determine the effect of rmTBI on spatial memory at both 2 weeks and 5 weeks after injury. Since gender and the interaction of treatment by gender had no significant effect on the parameters (such as escape latency, path length and so on) measured in the test (P ＞0.05), the data of male and female mice were combined together in the analysis in order to have a larger sample size. At 2 weeks after the injury, in the visible platform tests, TBI and control mice had similar escape latency (28.29 ± 3.28 and 21.97 ± 2.33s; P >

0.05; Fig. 8A) and path length (5.40 ± 0.65 and 4.47 ± 0.59 m; $P > 0.05$; Fig. 8B), which indicated that rmTBI did not affect mouse motility or vision. In the hidden platform-swimming test, TBI mice showed significant spatial memory deficits compared with the controls. The escape latency on the second, third and fourth day of the hidden platform test were longer (41.14 ± 2.84, 34.81 ± 2.42 and 28.83 ± 2.97 s) than those of the control mice (18.63 ± 2.07, 18.68 ± 2.43 and 15.38 ± 7.87 s; $P < 0.01$; Fig. 8C). The TBI mice needed to swim significantly longer distances to reach the platform (5.67 ± 0.58, 4.66 ± 0.39 and 4.20 ± 0.49 m) compared with control mice (3.25 ± 0.39, 3.17 ± 0.51 and 2.49 ± 0.51 m) on the second, third and fourth day ($P < 0.05$; Fig. 8D). In the probe trial on the last day of testing, the platform was removed. rmTBI significantly impaired spatial memory in the injured mice. The number of times the injured mice traveled into the platform zone, where the hidden platform was previously placed, was significantly less than that of the control (0.30 ± 0.15 and 2.40 ± 0.52; $P < 0.001$; Fig. 8E).

Even after 5 weeks of the injury, the deficit still persist in the TBI mice. As shown in Fig. 9A and 9B, in the visible platform tests, there was no difference between TBI and control in escape latency (29.56 ± 2.93 and 25.43 ± 3.52 s; $P > 0.05$) and path length (5.26 ± 0.60 and 4.99 ± 0.65 m; $P > 0.05$). In the hidden platform-swimming test, the escape latency on all the four days of the TBI mice were longer (40.46 ± 2.68, 38.18 ± 3.02, 31.36 ± 3.39 and 34.63 ± 2.47 s) than those of the control mice (28.36 ± 3.27, 20.93 ± 4.52, 18.51 ± 3.17 and 14.95 ± 2.13 s; $P < 0.01$; Fig. 9C). The TBI mice needed to

swim significantly longer distances to reach the platform (4.37 ± 0.55 m) compared with control mice (2.43 ± 0.37 m) on the fourth day ($P < 0.01$; Fig. 9D). In the probe trial, the TBI mice had less numbers of entries into the platform zone compared with control (0.78 ± 0.14 and 2.60 ± 0.50; $P < 0.01$; Fig. 9E).

3.6 Illumina whole genome gene expression assay

rmTBI activates complex biochemical cascades and alters the expression of different genes, whose products contribute to the pathophysiology seen following brain trauma.

After data normalization and background subtraction, of more than 45,200 gene probes (more than 30,800 genes) on the microarray, 19,735 probes (12,315 genes) in male and 21,252 probes (13,209 genes) in female met the inclusion criteria for detectable expression.

There is probe redundancy in the array platform that means two or more probes mapped to the same gene accession. In male TBI mice, a total of 89 probes (87 genes) were identified to be differentially expressed, and in female TBI mice, 240 probes (233 genes) were differentially expressed, either increased or decreased, compared to controls.

A further analysis found that in male mice, among the altered genes, 43 were up-regulated and 44 were down-regulated while in female mice, 84 were up-regulated and 149 were down-regulated (Fig. 10).

3.7 Functional classification

Those functionally characterized genes represented a diverse spectrum of biological processes, including apoptosis and cell cycle, metabolism (Ubiquitin-proteosome cascade

and energy metabolism), inflammation, angiogenesis and endothelial function, oxidative stress and NOS, transcriptional regulation, mRNA processing and protein metabolism, receptor and signal transduction, ion channel and transporter, membrane protein and intracellular trafficking and synaptic function and plasticity (Table 2 (female) and Table 3 (male)).

Chapter 4: Discussion

One of the most devastating consequences after moderate and severe TBI is the cognitive impairment, which greatly influences the quality of life of the patients. Cognitive deficits are also commonly seen after mild TBI. Not only has experimental and clinical data demonstrated that the hippocampus plays a critical role in learning and memory, studies also indicate that the hippocampus is uniquely vulnerable to injury following even mild brain trauma. The investigation of rmTBI is in its infancy, and while in vivo models provide essential information regarding behaviors and pathological and physiological sequelae on macroscopic and microscopic levels, we still need to address questions concerning dysfunction at the cellular and molecular levels. In the current study, we used the gene expression microarray to investigate the whole genome gene expression changes in mouse hippocampus after rmTBI. The underlying mechanisms of rmTBI appear to rely on suppression or up-regulation of a certain number of pathophysiologically relevant genes.

One aim of the present study was to modify and characterize successfully a model of rmTBI. After 25 daily applications of mild injury to the head, the injured mice exhibited transient neurological deficit, impaired weekly BWGR, changed behaviors in elevated plus maze, impaired spatial learning and memory in Morris water maze, all of which mimicked the manifestation occurred in the clinical form of rmTBI. Thus this paradigm can be reliably used to build rmTBI mouse model.

Microarray technology has provided opportunities to elucidate novel molecular and

cellular mechanisms that contribute to disease. Thus another aim of our research was to utilize this powerful genomic approach by applying genome wide differential expression analysis in the rmTBI mouse model.

To our knowledge, this is the first study to investigate the whole genome gene expression change following repetitive mechanical brain injury in vivo. There were few examples of in vivo studies in the literature determining the injury time points, therefore we injured mice every day for total 25 successive days.

Genes differentially expressed between control and rmTBI hippocampi were identified and functionally categorized. The products of those altered genes belong to a variety of cellular and molecular processes, including apoptosis, angiogenesis, intracellular signaling, oxidative stress, inflammation, cell skeleton, synaptic function and plasticity, and so on. We will discuss these functional categories one by one in the following section.

Before the start of any careful interpretation, it is worth noticing that in assigning the candidate genes to putative functional groups or pathways, it is not always possible to determine whether those functional groups or pathways are activated or inhibited from the direction of expression change. However, it seems clear that these mechanisms are being differentially modulated in response to rmTBI, and are thus of interest and worth further investigation. Previous studies have revealed very distinct, dynamic mechanisms in the cellular response to injuries of different severities (Klein and Ackerman, 2003; Tang et al., 1997). In those studies, the same gene was demonstrated to have different

patterns of expression depending on severity of injury and time point after injury. Therefore, the cellular functions or pathways that are altered after rmTBI rather than the individual genes identified in this study are considered targets for further investigation.

4.1 Apoptosis and cell cycle

Although moderate to severe TBI will cause necrotic and apoptotic cell death which is thought to contribute to altered behavior, a study showed that even mild traumatic brain injury also induce apoptotic cell death which is preceded by reduction in cellular Bcl-2 immunoreactivity (Raghupathi et al., 2002). The neuronal apoptosis in hippocampus has been reported to continue from weeks to over 1 year following TBI (Colicos and Dash, 1996; Smith et al., 1997). Since only a few hippocampus neurons with the characteristic morphological features of apoptosis can be detected at a given time (Colicos and Dash, 1996), the use of the whole hippocampus to extract RNA may explain why no genes were identified that are directly and specifically implicated in apoptosis (eg. TNF, Fas, P53, Bcl-2, et al). However, in the current study, we detected changes in several apoptosis-related genes.

In female TBI mice, some of those altered apoptosis-related genes are discussed as follows.

Dido1 (death inducer-obliterator-1), has been demonstrated to trigger apoptotic processes in vitro and a role in cell death during development (Garcia-Domingo et al., 1999).

Dusp8 is supposed to inactivate stress activated protein kinase SAPK/JNK and the

mitogen-activated protein kinase p38, and the repression of SAPK/JNK and p38 activation has an anti-apoptotic role (Javelaud and Besancon, 2001; Nagata and Todokoro, 1999).

Erp16 has been shown to play a role in endoplasmic reticulum induced apoptosis demonstrated by that overexpression of wild-type ERp16 inhibited endoplasmic reticulum stress-induced apoptosis whereas expression of inactive mutant increased the level of apoptosis (Jeong et al., 2008).

mtDNA-ND5, is the core subunit of the mitochondrial respiratory chain complex I and the inhibition of mitochondrial complex I is thought to induce apoptosis by inducing mitochondrial reactive oxygen species (ROS) production (Li et al., 2003). Another study also showed that mitochondrial generated ROS after transient brain ischemia and inhibition of mitochondrial complex I could prevent the formation of highly damaging hydroxyl radical after ischemia (Piantadosi and Zhang, 1996).

Pdcd4 (Programmed cell death protein 4), is one of the genes which have been found to be upregulated during apoptosis in several apoptosis-inducible cell lines like thymocytes, T cells, B cells, pheochromocytoma and vascular smooth muscle cells (Liu et al., 2010; Shibahara et al., 1995).

In male TBI mice, the apoptosis related genes include the following ones.

Ccnt1 is a major subunit of the transcription elongation factor p-TEFb and study showed that inhibiting p-TEFb blocked the transcription of its target genes as well as cellular proliferation and apoptosis induced by c-Myc (Kanazawa et al., 2003).

Sh2b1 is a signaling adaptor protein that has been shown to be necessary for the survival of sympathetic neurons. Overexpression of Sh2b1 reduces hydrogen peroxide-induced cell death in PC12 cells and hippocampal neurons (Lu et al., 2010b).

Tnfrsf21 (DR6) is a member of the TNF-receptor superfamily. Recent study indicated that amyloid precursor protein (APP) and DR6 are components of a neuronal self-destruction pathway in which an extracellular fragment of APP, acting via DR6 and caspase-6, contributes to Alzheimer's disease (Nikolaev et al., 2009). In addition, this receptor has also been shown to activate NF-kappaB and MAPK8/JNK, induce cell apoptosis, and may be involved in inflammation and immune regulation.

Thus, there were parallel incidences of both the promoted and inhibited expression of different anti-apoptotic and apoptosis-inducing genes in the hippocampus following rmTBI, as noted in the present study. The disorder between apoptotic and reparative mechanism may contribute to the pathogenesis after injury.

Recent evidence has suggested that one mechanism linked to the death of terminally differentiated neurons is aberrant re-entry into the cell cycle, and possible connections between oxidative stress and unscheduled cell cycle re-entry in some neurodegenerative disorders like Alzheimer's disease, Down syndrome, Parkinson's disease (Klein and Ackerman, 2003). In the current study, several genes whose products are involved in the regulation of cell cycle were found to be altered as a result of rmTBI.

In female TBI mice, those altered genes include the following ones.

Lin54 is a component of the LIN, or DREAM, complex, an essential regulator of

cell cycle genes and the complex can both act as a transcription activator or repressor depending on the context (Schmit et al., 2009).

Rpa1 is a subunit of replication protein A complex which is essential for DNA replication, recombination, and repair in eukaryotes (such as the initiation of cell cycle checkpoints following exposure to DNA replication stress). Rpa1-deficient cells demonstrated a slowing of S phase progression, G2/M cell cycle arrest, and apoptosis in HeLa cells (Dodson et al., 2004).

Tgfbr1 (Transforming growth factor, beta receptor 1) forms a heteromeric complex with type II TGF-beta receptors when bound to TGF-beta, transducing the TGF-beta signal to inhibit cell cycle in the G1 phase.

The inappropriate expression of cell cycle genes in postmitotic hippocampal neurons may serve as signals for death (Freeman et al., 1994; Park et al., 2000), leading to apoptosis rather than proliferation. Meanwhile, there are studies that demonstrated cell cycle inhibition could induce neuroprotection, reduce glial proliferation and scar formation after traumatic brain injury (Di Giovanni et al., 2005).

4.2 Metabolism (ubiquitin-proteosome cascade and energy metabolism)

The ubiquitin proteasome system (UPS) controls the turnover of innumerable cellular proteins. It targets misfolded or unwanted proteins for general proteolytic destruction and tightly controls destruction of proteins involved in development and differentiation, cell cycle progression, apoptosis and many other biological processes. Dysregulation of the UPS is believed to be both a cause and result of neurodegenerative

disease processes such as Alzheimer's, other frontotemporal dementias, Parkinson disease and so on.

In female TBI mice, the UPS related genes include: Nhlrc1, an E3 ubiquitin-protein ligase, suppresses the cellular toxicity of misfolded proteins by promoting their degradation through UPS together with EPM2A/laforin and HSP70 (Garyali et al., 2009).

Ube3b, a member of the E3 ubiquitin-conjugating enzyme family, has 75% sequence identity to the Oxi-1 protein of *C. elegans,* which was isolated in an experiment designed to identify oxidative stress-responsive genes. This homology suggests that UBE3B may play a protective role in either the classic stress response or in the stress response invoked by oxidative damage.

Ube2d2 encodes a member of the E2 ubiquitin-conjugating enzyme family and functions in the ubiquitination of p53 (Saville et al., 2004).

Znrf1 and Zubr1 are both E3 ubiquitin-protein ligase. Study showed that Znrf1 was associated with synaptic vesicle membranes in the presynaptic terminals and mutant Znrf1 inhibited Ca^{2+}-dependent exocytosis in PC12 cells (Araki and Milbrandt, 2003). This may suggest that Znrf proteins play a role in the establishment and maintenance of neuronal transmission and plasticity and dysregulation of them may result in abnormal accumulations of their target proteins and lead to abnormal signaling and disease. Zubr1, which is also called Ubr4, is an E3 protein that recognizes proteins bearing specific N-terminal residues that are destabilizing according to the N-end rule, leading to their ubiquitination and subsequent degradation (Tasaki et al., 2005).

In male TBI mice, Fbxo10 is a component of the SCF (SKP1-CUL1-F-box protein) complex which acts as an E3 protein-ubiquitin ligase. SCF-type ubiquitin ligases have been shown to regulate timely cell cycle progression (Vodermaier, 2004).

Stub1 is another E3 ubiquitin-protein ligase. In the senescence-accelerated mice (SAMP8), which is a suitable animal model to investigate the fundamental mechanisms of age-related learning and memory deficits, study revealed an up-regulation of Stub1 in the hippocampus of the SAMP8 mice but a down-regulation after treatment with a Chinese medicine that is found to have the ability to ameliorate age-related learning and memory deficits (Zhang et al., 2008a; Zheng et al., 2008). Other study showed that Hsp70/Stub1 played an important role in the pathogenesis of tauopathies by rescuing phosphorylated tau-induced cell death (Shimura et al., 2004).

Ubiquitin-proteasome system dysfunction may lead to abnormal protein deposition, mitochondrial failure and decreased expression of synaptic proteins. The remaining challenge is to identify the protein or proteins those genes target for rapid turnover, and to determine the roles of both gene products and their targets in rmTBI. The result in our study hints that protein degradation and quality control is one of rmTBI's effects on the brain. Regulation of USP levels or activity could provide a mechanism to alter the stability of key proteins.

As for the energy metabolism, in female TBI mice, the down regulation of Amy1 (salivary amylase 1) after injuries is of particular interest. Amy1 breaks down starch (such as from wheat, potatoes and rice, a major component of modern diet, comprising

40-60% of our calories) into glucose, which is the only energy source of the nerve cells. Besides, Amy1 has been considered as a marker of sympathetic nervous system (SNS) activity. During stress, SNS is responsible for the "fight or flight" response, which includes increased cardiovascular tone, faster breathing rate, and increased blood flow to muscles. The attenuation in the responsiveness of the SNS (indicated by down regulation of Amy1) in the face of chronic stress (rmTBI) may be protective from chronic energy expenditure.

Coq3 (coenzyme Q3 methyltransferase) is required for the 2 steps in the biosynthesis of coenzyme Q, which is a critical component of the electron transport pathways. Study also found that reduced levels of CoQ resulted in a progressive uncoordinated phenotype in *C. elegans* that was correlated with the appearance of degenerating GABA neurons (Earls et al., 2010).

Oat (Ornithine aminotransferase) is involved in the regulation of redox homeostasis, and the shifting of the redox balance has been implicated in the pathogeneses of several neurodegenerative diseases. In a study of mycotoxin-induced apoptotic cell death, Oat was found to be up-regulated in mouse hippocampal cells after treating with Ochratoxin A (Yoon et al., 2009).

Pde4b encodes a cAMP-specific, cyclic nucleotide phosphodiesterase. It was shown to localize in regions of the mouse brain associated with reinforcement, movement, and affect (Cherry and Davis, 1999) and it has been reported to play various roles in central nervous system (CNS) functions including memory and in diseases such as depression,

anxiety and schizophrenia (Siuciak et al., 2008; Zhang et al., 2008b).

St8sia5 is involved in the synthesis of gangliosides, which are found in high abundance in nervous tissue, particularly in synaptic membranes. Various studies have suggested a protective role of gangliosides after CNS injury (McIntosh, 1993), such as reducing behavioral deficits following brain injury by preventing secondary neuronal degeneration and/or enhancing structural reorganization of remaining afferents (Sabel et al., 1984).

In male TBI mice, Appl1, acting as a common downstream effector of AdipoR1 and -R2, mediates adiponectin-evoked endothelial NO production and endothelium-dependent vasodilation (Cheng et al., 2007). Appl1 also associates with TrkA and GIPC1 and is required for nerve growth factor-mediated signal transduction for the growth and survival of neurons (Lin et al., 2006). Adiponectin signaling through Appl1 is necessary to exert its anti-inflammatory and cytoprotective effects on endothelial cells. Reduction of Appl1 expression may thus suppress neurite outgrowth and decrease adiponectin-evoked endothelial NO production and endothelium-dependent vasodilation.

Coq7, or called as Clk1, its encoding protein is a mitochondrial hydroxylase that is necessary for the biosynthesis of ubiquinone. Clk1(+/)(-) mutant mice have enhanced resistance to neurological damage following global cerebral ischemia-reperfusion (I/R) injury. These mutants also have less oxidative damage resulting from ischemia and reperfusion (Zheng et al., 2010). Coq7 is thought to be a aging –associated gene and

reduction of its activity slows down aging in Caenorhabditis elegans and in mice (Wang et al., 2009). The increase expression of Coq7 in our study thus may partially underlie the pathology after rmTBI.

Other mitochondria related genes like Mrpl38 (mitochondrial ribosomal protein 38), mtDNA-ATP6 (mitochondrially encoded ATP synthetase 6) and Slc25a38 (a mitochondrial carrier) are also found to be altered following rmTBI in male mice. These genes may play a compensatory role in the face of mitochondrial dysfunction, or alternatively may contribute to pathogenesis.

As discussed above, in both male and female mice, most of the genes related to energy metabolism have been shown to be altered toward the pathogenic direction. It seems reasonable, then, to suggest that a therapeutic effect in rmTBI might be obtained by enhancing the function of energy metabolism to block development of damages following the injury.

4.3 Inflammation

Inflammation has both beneficial and detrimental effects (Bethea, 2000). Several studies revealed that traumatic CNS injury could trigger severe systemic effects that lead to inflammation and pathological autoimmunity (Donnelly and Popovich, 2008; Popovich and Jones, 2003). In agreement with previous reports, our analysis found up-regulations of some inflammatory genes.

In female TBI mice, the inflammation-related genes include the following ones.

Tafa2, whose product belongs to a secreted family with conserved cysteine residues

and restricted expression in the brain. It is distantly related to MIP-1alpha, which is a neutrophil chemoattractant leading to acute neutrophilic inflammation. MIP-1 alpha also induces synthesis and release of other pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6 and TNF-alpha from fibroblasts and macrophages (Tom Tang et al., 2004).

Dab-2, whose expression has been shown to increase significantly in the rat brain following experimental cryoinjury in relation to CNS inflammation (Moon et al., 2005).

Dpp8 with a role in T-cell activation and immune function was also increased after the injury.

Sdc4, whose mRNA is distributed in glial cells of the forebrain, especially in the hippocampus, has been shown to be increasedly expressed involved in brain injury and may provide a supportive environment for regenerating axons in concert with heparin-binding growth factors (e.g. FGF and PTN) in the injured brain (Iseki et al., 2002).

Meanwhile, those anti-inflammatory and neuroprotective genes showed decreased expressions. Down-regulation of inflammation is important for the limitation of tissue injury at later stages of lesion development. The decreased anti-inflammation related genes in female include: Usp52 can modulate NF-κB induction and control inflammatory responses (Fiorentino et al., 2002).

Fbxl11 encodes a member of the F-box protein family and overexpression inhibits NF-κB activity (Lu et al., 2010a) while another decreased gene Irak1 is partially responsible for IL-1-induced increase of NF-κB.

Ikbkb can also trigger NF-κB signaling, which has dual roles in both tissue protection and systemic inflammation.

Products of Pou2f1 and Sharpin are both involved in normal immune development and control of inflammation.

In male TBI mice, it is not surprising that Lcp1 (actin-bundling protein L-plastin) has an increased expression after rmTBI since it has been shown that L-plastin phosphorylation is involved in integrin activation, which is a crucial aspect of inflammation and immunity (Jones et al., 1998).

Another up-regulated gene in male injured mice is Neu1, which has been found to regulate Toll-like receptor (TLR) activation (Fu and Gao, 2009). TLRs play an important role in the pathophysiology of infectious diseases, inflammatory diseases, and possibly in autoimmune diseases.

Finally, another gene that was altered in male injured mice is Errfi1. It is an immediate early gene that is transcriptionally induced by a divergent array of extracellular stimuli and has a possible role in the response to persistent stress (Makkinje et al., 2000).

Both the male and female data set demonstrated a predominantly inflammatory response, as the majority of pro-inflammatory genes were up-regulated and anti-inflammatory genes were down-regulated. A critical aspect of the inflammatory response is the ability to stop the inflammation, referred to as the resolution phase, an active process involving expression of anti-inflammatory agents. Such over inflammation

can be detrimental if left unchecked and may contribute to the poorer outcome of the rmTBI mice compared with the control.

4.4 Angiogenesis and endothelial function

The neurovascular (NV) unit is composed of functionally integrated cellular (including brain endothelial cells, astrocytes, pericytes, and smooth muscle cells) and acellular elements that form the basement membrane. After brain insults such as traumatic brain injury or hypoxia or ischemia, there will be dramatic changes in the NV unit including disruption of tight junctions between endothelia, breakdown of the basal lamina, and endothelial proliferation, migration, and reorganization to form new capillaries and microvessels. Angiogenesis is thought to be required to re-establish metabolic support.

Only in female TBI mice there are altered genes related to angiogenesis and endothelial function. The related genes include the following ones.

Col4a1 encodes the major type IV alpha collagen chain of basement membranes and it has been found to possess the anti-angiogenic activity and specially inhibits endothelial cell proliferation, migration and tube formation. Thus the decreased expression of the above angiogenesis inhibitors may be consistent with increased angiogenesis in the mouse brain after rmTBI.

Flt1 encodes a member of the vascular endothelial growth factor receptor (VEGFR) family to regulate VEGF signaling. Study revealed that neutralization of Flt1 receptor function resulted in significantly decreased astroglial mitogenicity and scar formation and

some increase in endothelial degeneration (Krum et al., 2008). Thus neutralization of Flt1 provides the possibility to inhibit astrocyte proliferation while without decreasing angiogenesis which is essential to wound repair.

The product of Ogn is a proteoglycan and there is a study showing that downregulation of Ogn is necessary for proper arteriogenesis, which is able to compensate for the stenosis of major arteries. After brain injury, upregulated Ogn may not be sufficient for the increased need of blood supply for the metabolism of the injured brain. Further studies using Ogn knockout mice with rmTBI may help in understanding how this glycoprotein influences the mechanical properties of the vascular wall during adaptive growth after brain injury.

Other altered genes such as Ptprb, Mmp16, Ndrg1 and so on have also been shown to play important roles in blood vessel remodeling, myelin synthesis and lipid metabolism (Lachat et al., 2002; Lafleur et al., 2002; Okuda et al., 2004).

Most of the expression changes of the angiogenesis-related genes following rmTBI are consistent with the general notion that increased angiogenesis will occur during wound healing. While Ogn is of special interest but will need further research to establish its possible influence on the pathogenesis of brain injury.

4.5 Oxidative stress and NOS

The crucial role of oxidative stress and lipid peroxidation has been demonstrated in many neurological disorders, including neurodegenerative diseases, mental disorders, stroke, brain traumas and so on. Oxidative stress gives rise to the production of nitric

oxide and superoxide, which are themselves highly reactive but can also combine to form a highly toxic anion, peroxynitrite. The toxicity of these free radicals results from their modification of macromolecules, especially DNA, and their induction of apoptosis.

In female TBI mice, Atic is one of the intriguing genes which has been observed to have anti-inflammatory/anti-oxidant and neuroprotective functions following treatment of glial cells with LPS and Abeta peptide (Ayasolla et al., 2005). The dramatic downregulation of Atic after rmTBI may thus impair reparative ability following injury and contribute to the pathogenesis of brain injury.

Dnajc18 encoding the homolog of heat shock protein 40 was found to be upregulated after the injury. Hsp40 has been shown to be synergistic induced together with HSC70 in the mouse hippocampal neurons after cerebral ischemia (Tanaka et al., 2002) and several studies have demonstrated the protective effects of Hsp40 in several animal models of neurodegenerative diseases (Muchowski and Wacker, 2005). Since evidence is accumulating that heat shock proteins are protective against brain damage like ischemia and studies have placed more and more emphasis on the crucial role of the interaction of several stress proteins for molecular chaperone activity, future studies will be needed to clarify the association and functional role of those Hsp after brain injury.

Another protective gene that is upregulated is Gstm2, glutathione S-transferase mu 2, which is thought to play a role in detoxification of products of oxidative stress (Chanas et al., 2002).

Sulfiredoxin 1 (Srxn 1) is an enzyme responsible for reducing hyperoxidized

peroxiredoxins, a cysteine-based antioxidant enzyme excerting a neuroprotective effect in several models of neurodegeneration. Upregulation of Srxn 1 may represent an intrinsic antioxidant defense of the brain after injuries (Papadia et al., 2008; Soriano et al., 2008).

NOS3 is endothelial NO synthesase whose activation is shown to be neuropretective after ischemic brain damage mediated by the vasodilation effect of NO despite the effects of NOS1 and NOS2 activity after ischemia are detrimental (Love, 1999). Thus the dramatic downregulation of NOS3 after rmTBI may exacerbate the pathogenic consequence.

In male TBI mice, the oxidative related genes were all upregulated. These genes include the following ones. Cyb5r1, its product is believed to be associated with the oxidative state of cells.

Erp29, is involved in the processing of secretory proteins within the endoplasmic reticulum (ER) and has been shown to take part in the ER stress signaling (Zhang and Richardson, 2011).

Gstz1, encodes a phase II detoxifying enzyme that provides protection against oxidants and cellular toxins (Maeda et al., 2005)

The last candidate gene in this category is Mapk8ip1, also called Jip-1. This scaffolding protein causes inhibition of JNK-regulated activity by tethering JNK in the cytoplasm and inhibiting JNK phosphorylation of c-Jun. Some studies have revealed the co-localization of the Jip-1 with APP, JNK and hyperphosphorylated Tau in the neurofibrillary tangles of AD and b cells of type II diabetes patients (Beeler et al., 2009).

This may suggest that mechanism involving Jip-1 and the JNK pathway can trigger neuronal and islet degeneration.

In all, the expression changes of these oxidative related genes may point out that rmTBI could trigger oxidative stress responses in the hippocampus, leading to consequent neural dysfunction. Although the intrinsic anti-oxidative pathways were initiated as shown by the upregulation of those anti-oxidative genes, further studies need to be designed to test the precise molecular mechanisms of oxidation and anti-oxidation in the pathophysiology following the injury.

4.6 Cytoskeleton

Neurofilaments are the major cytoskeletal components of axons while dendrites contain a proportionately greater number of microtubules. Loss of cytoskeletal proteins via proteolysis has been observed following moderate to severe traumatic insults. Even in models that produce mild brain injury loss of cytoskeletal proteins has been demonstrated in the absence of significant cell death (Saatman et al., 1998).

In female TBI mice, those downregulated cytoskeleton-related genes and their functions are as follows.

Kank3 has been shown to negatively regulate the formation of actin stress fibers, which form a specific cytoskeletal organization of actin monomers and are involved in cell growth and cell movement (Zhu et al., 2008).

Crocc encodes the protein rootletin that could be found in a variety of neurons such as granular neurons in the dentate gyrus. Both overexpression and depletion of rootletin

have an effect on centrosome cohesion before mitosis (Bahe et al., 2005).

Frmd4a is a scaffolding protein controlling the activation of Arf6, which is a central player in actin cytoskeleton dynamics and membrane trafficking during junctional remodeling and epithelial polarization (Ikenouchi and Umeda, 2010).

Ssh3 encodes a protein phosphatase that plays a critical role in regulating actin cytoskeletal reorganization by dephosphorylating cofilin (Ohta et al., 2003).

In contrast, after the injuries, expression levels of some of the cytoskeleton related genes in female mice were upregulated. These genes are listed here.

Beta-Actin is one of the two nonmuscle cytoskeletal actins and is a highly conserved protein involved in cell motility, structure and integrity. The increase of Actb after rmTBI is consistent with the previous finding that after oxidative stress, some cytoskeleton components including actin appeared to increase over time, which is also supported by other studies demonstrating that cytoskeletal proteins contribute to the dynamic blood–brain barrier responses (Ning et al., 2011).

Alpha-Parvin belongs to a family of proteins involved in linking integrins and associated proteins with intracellular pathways that regulate actin cytoskeletal dynamics and cell spreading, motility and survival (Sepulveda and Wu, 2006).

Tubb2b encodes β-tubulin 2B and it has been proposed to be a crucial effector of neuronal polarization and axonal growth and guidance (Jaglin and Chelly, 2009).

In male mice treated with TBI, the cytoskeleton related genes include the following ones.

LOC433749, a predicted gene, was increased after the injury. The product of this gene putatively belongs to the RhoA family of GTPase, which is known to regulate actin cytoskeleton dynamics and formation of stress fibers. In neurons, activation of RhoA pathway inhibits the growth and repair of neural pathways and axons whereas RhoA inhibition was found to improve re-myelination.

Mtap6 (microtubule-associated protein 6) was downregulated after rmTBI. Microtubule-associated proteins (MAPs) bind to cytoskeletal microtubules and stabilize their assembly. Stabilized microtubules are thought to be essential for neuronal development, maintenance, and function. Studies revealed that Mtap6 has a particular relevance to schizophrenia pathology, since Mtap6$^{-/-}$ mice showed synaptic defects, including depleted synaptic vesicle pools and impaired synaptic plasticity, associated with severe behavioral impairments (Eastwood et al., 2007).

Syne1 encodes a protein which specifically localized to a postsynaptic endocytotic zone of excitatory synapses. RNAi-mediated Syne1 knockdown has been shown to affect the internalization of synaptic proteins, including glutamate receptors, which suggests that it contributes to the capacity for postsynaptic plasticity inherent to excitatory synapses (Cottrell et al., 2004).

These alterations are consistent with the activation of glial cells, enhanced neurogenesis in the dentate gyrus and extracellular matrix remodeling, and disrupted synaptic function which have been reported to occur following brain injury (Dash et al., 2001).

4.7 Transcriptional regulation, mRNA processing and protein modifications

Transcriptional initiation of a gene requires combinatorial interactions between sequence-specific transcription factors (TF) and their regulatory factors as well as the remodeling of chromatin structures to facilitate RNA polymerase access. In the current study, a large part of the altered genes belong to TFs or coordinators of transcriptional activity of many TFs.

In female TBI mice, these genes include, Rtel1 (Regulator of telomere length, playing a central role DNA repair and in the maintenance of genomic stability), Xrcc1 (whose product is involved in the efficient repair of DNA single-strand breaks by participating in the base excision repair pathway) , Zbtb38 (whose product acts as a transcriptional activator and may be involved in the differentiation and/or survival of late postmitotic neurons), Nacc1 (its protein functions as a transcriptional corepressor in neuronal cells through recruitment of Histone deacetylase 3 and 4.) and so on.

Most of the altered genes related to transcriptional regulation were downregulated and those genes are either involved in either transcriptional activation or inhibition. Thus further investigation of their down stream effectors may aid to elucidate the exact mechanism of transcriptional regulation in response to rmTBI.

In male TBI mice, the expression changs of genes involved in transcriptional regulation were also observed. For example, the transcription of Cited2 (a CBP/p300 interacting transactivator) previously reported to be induced by cytokines in immune cells. Moreover hypoxia inducible factor (HIF-1) was found to be in the upstream

transcriptional control of it. Studies also observed that the expression of Cited2 was also increased following transient forebrain ischemia (Sun et al., 2005). In another study, cited2 has been shown to signal through peroxisome proliferator-activated receptor-gamma to regulate death of cortical neurons after DNA damage (Gonzalez et al., 2008). The increased expression of Cited2 after rmTBI further supports the idea that Cited2 appears to be related to inflammatory, neuronal death and neurodegenerative processes evoked by aberrant neuronal activation/damage.

The product of Myst2 (also called Hbo1) has a histone H4-specific acetyltransferase activity and is required for global histone H4 acetylation, steroid-dependent transcription and DNA replication and transcription. A recent study showed that physiological stress conditions that activate p53 (eg. hyperosmotic shock and DNA replication fork arrest) inhibit Hbo1 histone acetyltransferase activity in a p53-dependent manner (Iizuka et al., 2008). Whether inhibited Hbo1 activity will upregulate the expression of Hbo1 in a negative feedback manner needs further investigations.

Rreb1 encodes a zinc finger transcription factor that binds specifically to RAS-responsive elements (RREs) of gene promoters and it has been shown to be involved in a lot of genes' transcriptional regulation. For example, enhancing calcitonin expression, repressing the angiotensinogen gene, negatively regulating the transcriptional activity of AR, potentiating the transcriptional activity of NEUROD1, transactivating p53 expression upon exposure to genotoxic stress and so on.

Following transcription, the pre-mRNA is then processed by splicing out the introns

to form mRNA. Alternative splicing is an important mechanism for expanding proteome diversity from a limited number of genes and it is determined by the nuclear concentrations of different proteins which are altered by physiological stimuli or phosphorylation state (Xie et al., 2003). Meanwhile, mRNA transport and local translation are critical for synaptic plasticity mediated by activity or experience and memory. Aberrant pre-mRNA processing has long been suggested to contribute to neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) (Lin et al., 1998) possibly due to rapid degradation of pre-mRNA which results in loss of proteins and their activities.

In female TBI mice, those altered genes related to mRNA processing include the following ones.

Nova2, is a significantly downregulated gene after rmTBI. Nova proteins are evolutionary conserved neuron-specific splicing factors that are expressed specifically in the CNS. A previous study reported that a muscarinic agonist, but not the glutamatergic agonist, induced a significant downregulation of Nova2 mRNA in rat's striatum (Jelen et al., 2010). Furthermore, Nova2 was necessary for the induction of slow inhibitory postsynaptic currents in hippocampal neurons (Huang et al., 2005).

Syncrip encodes an RNA-interacting protein which is suggested to be important for the stabilization of mRNA. Studies also suggested that Syncrip was transported within the dendrite and raised the possible role of Syncrip in the mRNA turnover and the regulation of local protein synthesis in neuronal dendrites (Bannai et al., 2004).

In male TBI mice, those altered genes related to mRNA processing include the following ones.

Lrpprc, has been suggested to have a specific role in mt-mRNA homeostasis. In a research that used short hairpin RNA (shRNA)-mediated graded knockdown of Lrpprc to study mitochondrial and nuclear responses to loss of Lrpprc systematically, researchers found all mtDNA-encoded mRNA transcripts decrease in proportion to the loss of Lrpprc (Gohil et al., 2010).

Hnrpl, encodes a component of the heterogeneous nuclear ribonucleoprotein (hnRNP) complexes that provide the substrate for the processing events that pre-mRNAs undergo before becoming functional, translatable mRNAs in the cytoplasm.

Rnasen encodes a member of the ribonuclease III superfamily of double-stranded (ds) RNA-specific endoribonucleases and is the core nuclease that executes the initiation step of microRNA (miRNA) processing in the nucleus (Lee et al., 2003). MicroRNAs participate in the maintenance of adult neural cell traits, promote cellular homeostasis, dampen endogenous and exogenous stress responses, and modulate multiple parameters that are associated with synaptic plasticity. Since recent evidence suggests that microRNAs may be a contributing factor to neurodegeneration as seen in Alzheimer's diseases (Yokota, 2009), future research on mircoRNAs and the effect of altered Rnasen on mircoRNAs will have significant importance in the pathophysiological process of rmTBI.

Following transcription, modifications at protein level is also an important way to

control the amount and function of proteins. In female TBI mice, the altered genes related to protein level regulation and their functions are as follows.

Furin encoding protein is a calcium-dependent serine endoprotease and has the ability to cleave the beta-site APP cleaving enzyme propeptide domain to form the mature enzyme. (Bennett et al., 2000).

Sgpp1 catalyzes the degradation of Sphingosine-1-phosphate (S1P) and studies have demonstrated a functional role of S1P synthesis and receptor expression in astrocyte proliferation leading to astrogliosis during the terminal stages of neurodegeneration (Wu et al., 2008).

Msl2 encodes a RING finger protein that is required for X chromosome dosage compensation in Drosophila males and ectopic expression of it in females resulted in decreased viability (Kelley et al., 1995). A recent study showed that Msl2 can promote ubiquitin-dependent cytoplasmic p53 localization which is thought to play an active role in p53-mediated functions such as apoptosis and autophagy (Kruse and Gu, 2009). Thus it is of interest to further study the function and consequence of upregulated expression of Msl2 in females after rmTBI.

In male TBI mice, the altered genes related to protein level regulation and their functions are as follows.

Eif3i, encodes a component of the eukaryotic translation initiation factor 3 (eIF-3) complex, which is required for the initiation of protein synthesis. There has been a study that showed overexpression of eIF-3 was at least partially responsible for cadmium

carcinogenesis (Joseph et al., 2004). It is possible in our study that the overexpression of eIF-3 might have resulted in translational up-regulation of specific genes alleviating or aggravating the pathophysiology after rmTBI; and this, in turn, results in, at least in part, the pathogenesis following the injury.

Sulfotransferase 1a (Sult1a1) specifically catalyzes the sulfonation of the catecholamines, dopamine, adrenaline and noradrenaline as well as of drugs such as apomorphine. It has been reported to play a role in anti-oxidation and mood state (Conti et al., 2007).

4.8 Receptor and signal transduction

Trafficking of receptors into and out of synapses plays a critical role in the synaptic plasticity. Synaptic strength can be altered by changes pre-synaptically by the release of neurotransmitters or post-synaptically by alterations in the number or sensitivity of neurotransmitter receptors.

In female TBI mice, those altered genes related to receptors are partially discussed as follows.

Pkd1 encodes Trpp1, a member of transient receptor potential (TRP) channel superfamily in vascular endothelial cells. It has been found that dysfunction or dysregulation of TRP channels impaired endothelium-dependent vascular relaxation, and Trpp1 knockout mice displayed vascular fragility, edema, and localized hemorrhages (Muto et al., 2002). Therefore the dysregulation of Pkd1 may result in endothelial dysfunction and contribute to the progression of pathological events after rmTBI.

Grik5 encode a kainate receptor (KA2) that is a member of the ionotropic class of glutamate receptors, which also includes NMDA and AMPA receptors. Studies knocking down the expression of KA2 showed suppressed assembly of the GluR6/KA2-PSD95-MLK3 signaling module, inhibited JNK activation and increased neuronal survival in CA1 region after 5 days of ischemia/reperfusion (Jiang et al., 2007). The downregulation of excitatory glutamate receptor gene expression may have a neuroprotective role after rmTBI.

Igf1r encodes insulin-like growth factor 1 (IGF-1) receptor and in the brain, IGF-1 signaling promotes neuronal survival, neurite outgrowth, maturation of oligodendrocytes and myelination (D'Ercole et al., 1996). One study showed that following weight drop injury in rats, no change of Igf1r mRNA was observed from 1 to 7 days, despite an increase of Igf1 mRNA (Sandberg Nordqvist et al., 1996). While other studies found increased Igf1r after cerebral ischemia and contusive brain injury in rats and increased Igf1 in human respectively (Bergstedt and Wieloch, 1993; Madathil et al., 2010; Wildburger et al., 2001). In our current study, the mRNA of Igf1r was decreased after rmTBI with no change of Igf1 mRNA. This discrepancy may result from the different animal models that were used or different time points after the injury when tested.

In male TBI mice, decreased expression of Gria2 is the only change observed in this category after rmTBI. Gria2 encodes one of AMPA subunits GluR2. AMPA receptors mediate fast excitatory synaptic transmission in the CNS and play a key role in hippocampal synaptic long-term potentiation (LTP) and depression (LTD).

In both females and males TBI mice, there are genes encoding one of the glutamate receptors (Kainite and AMPA respectively) showed decreased expression after rmTBI. After injury, the levels of interstitial glutamate increase, contributing to a wide variety of TBI-related oathologies, including neurotoxicity, altered plasticity and memory deficits. Once released, glutamate binds to one of there different classes of receptors, NMDA, AMPA/Kainite and metabotropic, to initiate its biological effects. Thus the down-regulation of these receptor mRNAs may be an intrinsic protective mechanism to attenuate further damage by reducing signaling. Alternatively, decreased levels of these receptors may impair synaptic transmission and could contribute to hippocampal dysfunction. The result from the current study is consistent with other studies indicating that inhibition of AMPA-type glutamate receptors following TBI may protect against secondary damage only when administered within 10 hours post-injury (Bernert and Turski, 1996; Ikonomidou and Turski, 1996). This may result from a down-regulation of its target receptors as a result of the decreased mRNA levels we observed. Similarly to the case for the NMDA receptors, no changes in the mRNA levels of dopaminergic and cholinergic receptors were detected.

There are also genes related to signal transduction altered after the injury.

In female TBI mice, those genes are discussed as follows.

Mpp3, is dramatically elevated after rmTBI. It is a membrane-associated guanylate kinase playing an important role in signal transduction. Study showed that Mpp3 is a major PDZ-binding partners of the serotonin 2C (5-HT2C) receptor and prevents

desensitization of the 5-HT2C receptor in response to Ca^{2+} (Gavarini et al., 2006).

Fa2h, its product produces 2-hydroxylated fatty acids for incorporation into 2-hydroxydihydroceramide and 2-hydroxyceramide. These ceramide species in turn serve as precursors for the synthesis of galactosylceramides and sulfatides, the essential lipid components of normal myelin. A critical role of Fa2h in normal myelin maintenance is supported by that in human neurological diseases, the anomalous myelin produced in Fa2h mutations might alter CNS iron homeostasis by disrupting myelin integrity. Another role for Fa2h lies in lipid signal transduction and ceramide-mediated modulation of synuclein metabolism has been related to neurodegenerative disease (Jana et al., 2009; Stoica et al., 2005).

GTP-binding proteins (or G-proteins) are involved in various cellular processes, including growth responses, morphological changes and receptor signaling. The microarray analysis in the current study revealed that in female mice, rmTBI increased the mRNA levesl for Rragb (Ras-related GTP binding B) and Gpr155 (G protein-coupled receptor 155), suggesting enhanced intracellular signaling. Ther is evidence to suggest that Gpr155, an integral membrane protein related to G-protein coupled receptors, has been dysregulated in the caudate nucleus of Huntington's (HD) disease patients and HD animal models (Brochier et al., 2008; Hodges et al., 2006) and in lymphoblastoid cells of humans with autism spectrum disorders (Nishimura et al., 2007). A recent study showed that the expression pattern of Gpr155 mRNAs in mouse striatum was very similar to that of CB1 receptors (cannabinoid receptor) in rat. Many neurons that are identifiable as

GABAergic might express Gpr155, implicating its important role in GABAergic neurotransmission (Trifonov et al.).

In male TBI mice, the signal transduction related gene found to be altered after rmTBI is Zranb1, a positive regulator of the Wnt signaling pathway that acts by deubiquitinating APC tumor suppressor protein, a negative regulator of Wnt-mediated transcription (Sowa et al., 2009; Tran et al., 2008). Study investigating the relationship between Wnt signaling and experience-related regulation of synapse numbers and mossy fiber connectivities in the adult hippocampus showed that inhibiting Wnt signaling suppressed the effects of enriched environment on synapse numbers and further reduced synapse numbers in control mice (Gogolla et al., 2009). Thus the downregulation of Zranb1 may exacerbate rmTBI outcome by the modulation on synapses.

In all, rmTBI may dysregulate several receptors expression and disturb signal transductions which thus contribute to pathophysiology after the injury.

4.9 Ion channel and transporter

The microarray analysis revealed that the mRNA levels for several ion channels and transporters were altered in the hippocampus following rmTBI.

In female TBI mice, those altered genes are discussed as follows.

Slc17a7 encodes a vesicle-bound, sodium-dependent phosphate transporter and its product functions in glutamate transport by mediating the uptake of glutamate into synaptic vesicles at presynaptic nerve terminals of excitatory neurons. Since it plays the important role of regulating concentrations of glutamate in the extracellular space,

keeping it at low levels, its decreased expression may result in neuronal excitotoxicity and finally lead to neurodegenerative pathologies.

Genes encoding two amino acid transporters, Slc7a14 and Slc7a8, were found to be up and down regulated respectively following the injury. Slc7a14 is the youngest family member of Cationic Amino Acid Transporters (CATs) with yet unknown function, although it is highly expressed in the CNS while has virtually no expression in the periphery (Sreedharan et al., 2010). Slc7a8 encodes a L-type amino acid transporter called Lat2. Studies indicated that Lat2 mediates arginine efflux in exchange with glutamine (Broer et al., 2000) and it also medicates glutamine efflux from cultured astrocytes (Deitmer et al., 2003). Glutamate, once released into the synaptic cleft, is largely recycled by the glutamate-glutamine cycle, which involves uptake into astrocytes, conversion into glutamine and subsequent release of glutamine from astrocytes as a precursor for neuronal glutamate synthesis. The results from previous studies may suggest that Lat2 might play a significant role in the recycling of glutamate in the brain.

Slc8a2 encodes a Na^{+}/Ca^{2+} exchanger locating on the plasma, mitochondrial and endoplasmic reticular membranes of excitable cells. Under normal physiological conditions, Na^{+}/Ca^{2+} exchangers export Ca+ from the cell. However, under cellular stress, such as excitotoxicity, they can switch to 'reverse mode', which has a protective effect. The decreased expression of Slc8a2 may be associated with weakened neuroprotective ability and exacerbate the outcome following rmTBI.

Furthermore, Tpcn1, encoding one of the major voltage-gated calcium channels and

Nipa1, encoding a magnesium transporter in a variety of neuronal and epithelial cells were found to be downregulated and upregulated respectively after the injury. These observations add to the notion that rmTBI will trigger a wide range of pathophysiological changes in the hippocampus and this may further affect neuronal functions and synaptic plasticity.

In male TBI mice, the expressions of two voltage-gated potassium channels (Kcna2 and Kcnma1) were significantly downregulated following the injury. Voltage-gated potassium channels are involved in a wide variety of physiological processes, including neuronal excitability, and the downregulation of their mRNAs is likely to lead to decreased protein levels, impaired neuronal repolarization, and hyperexcitability.

Furthermore, a voltage-gated sodium channel (Scn2a1) was found to be downregulated after rmTBI. Alterations of these proteins may have implications in neuronal excitability and play a role in the posttraumatic epilepsy which is often observed following TBI (Annegers and Coan, 2000).

Following rmTBI, the glutamate recycling was inhibited which made the hippocampal neurons more susceptible to excitotoxicity and transportation of sodium, potassium, calcium and magnesium were disturbed which may disrupt the ionic homeostasis, leading to dysfunction of neuronal physiological processes.

4.10 Membrane protein and intracellular trafficking

In female TBI mice, the altered genes related to membrane protein and intracellular trafficking are discussed as follows.

Caln1 encodes calneuron 1, a member of the calmodulin superfamily, is expressed only in the brain with high expression in the hippocampus and cerebral cortex, the main structures essential for learning and memory. Ca^{2+} is a second messenger controlling many biological processes via interaction with a large number of Calcium-binding proteins like calmodulin. In the current study, downregulation of Caln1 expression may impair neuronal functions like learning and memory through decreasing Ca^{2+} signaling.

Cldn5 was also downregulated as a result of rmTBI. Its product belongs to claudin family, which is an integral membrane protein and a component of tight junction strands. A study in the rat cortical cold injury model found that during blood brain barrier (BBB) breakdown following injury, claudin-5 expression was significantly decreased, which suggests that claudin-5 could have an important role in BBB breakdown and could be a potential therapeutic target in the control of early brain edema (Nag et al., 2007). In a study of transient focal ischemia model, there was significant edema formation and the vascular tight junction protein claudin-5 underwent extensive degradation in the peri-infarct area after injury. By treating with the lipoxygenase inhibitor, degradation of claudin-5 was partially prevented and brain edema was significantly ameliorated. This may suggest that claudin-5 is involved in the brain microvasculature change contributing to the pathogenesis following brain injury (Jin et al., 2008).

Kifc2, the molecular motor in neurons, directs cargos to the plus ends of microtubules where the translation of specific mRNA transported may modify synaptic plasticity. Because the microtubules are arrayed with their plus ends extending into

dendrites, the KIFs have long been thought to direct RNA-containing cargos into these processes (Hanlon et al., 1997; Saito et al., 1997). Thus the dysregulation of it after rmTBI may interfere with normal synaptic transportation and then impair synaptic plasticity.

In male TBI mice, the genes related to membrane protein and intracellular trafficking are discussed as follows.

Bzrap1 encodes a protein which is prevalent in the murine mesolimbic system, specially abundant in the CA1 subfield of the hippocampus and specifically interacts with the peripheral benzodiazepine receptor (Zhou et al., 1997). In the subsequent study of Bzrap1, the expression level of Bzrap1 was significantly elevated after longterm treatment with neuroleptic haloperidol, repeated electroconvulsive shock administration and several classes of antidepressant. These results suggested that it is one of the common functional proteins up-regulated after chronic antidepressant treatment and might have a role in the pathophysiology of affective disorders such as depression (Lee et al., 2010).

Rph3a encodes an effector of Rab3A which has been suggested to be a key molecule in modulating the levels of neurotransmitter release in neurons through either recruitment of synaptic vesicles or, more likely, Ca^{2+}-triggered membrane fusion. It was proposed that presynaptic vesicles might be guided to the vicinity of sites of exocytosis via the interactions between Rph3A-CASK-beta-neurexins (Zhang et al., 2001).

Gdi1 encodes GDP dissociation inhibitor 1, a regulator of one of the Rab small G

proteins (Rab3A) implicated in neurotransmission. A study using Gdi1-deficient mice demonstrated that Gdi1 had an important role in vivo for suppressing hyperexcitability of the CA1 pyramidal neurons because Gdi1-deficient neurons permitted a breakthrough of this hyperexcitable state leading to epileptic seizures (Ishizaki et al., 2000). Thus the downregulated Rph3a and Gdi1 expressions may lead to dysregulated exocytosis of neurotransmitters which is fundamental to synaptic neurotransmission.

4.11 Synaptic function and plasticity

The storage and retrieval of memories has been postulated to be represented by a vastly interconnected synaptic network in the brain, therefore, synaptic plasticity have very important roles in learning and memory.

In female TBI mice, as a result of rmTBI, the altered genes involved in synaptic plasticity include the following ones.

Crtc1 encodes cAMP-response element binding protein (CREB) regulated transcription coactivator 1 which regulates the expression of specific CREB-activated genes. Activity-dependent gene expression mediating changes of synaptic efficacy have been thought to be important for memory storage, but the mechanisms underlying gene transcriptional changes are poorly understood. In a recent study, the researchers observed that in neurons from AD transgenic mice, Crtc1 played a key role in coupling synaptic activity to gene transcription required for hippocampus dependent memory and they suggested that A_β could impair cognition in AD by affecting Crtc1 function (Espana et al., 2010).

Ctnnd2 encodes delta-catenin which is down-regulated during neuronal migration and locates in dendrites in adults neurons. Thus delta-catenin is thought to be fundamental for the establishment and maintenance of dendrites and synaptogenesis. In a study aiming to understand the function of presenilin 1 (PS1), the researchers found that delta-catenin may function as an interactor with presenilin 1 (Zhou et al., 1997), whose mutation causes early-onset familial Alzheimer's disease.

Dgkb encodes diacylglycerol kinase (DGK), beta, and DGKs are regulators of the intracellular concentration of the second messenger diacylglycerol (DAG) thus play a key role in a wide range of cellular processes. A genetic variant of Dgkb has recently been identified to be associated with late-onset Alzheimer's disease in Caribbean Hispanic individuals (Lee et al., 2010).

In male TBI mice, Camkk2 encodes calcium/calmodulin-dependent protein kinase kinase beta and was found to be dramatically downregulated following rmTBI in the current study. The calcium/calmodulin (CaM) kinase cascade regulates gene transcription, which is required for long-term memory formation. In a study using Camkk2 null mice, the researchers found that in male but not female mice Camkk2 was required for spatial memory formation and for activation of the transcription factor CREB in the hippocampus by spatial training (Mizuno et al., 2007; Peters et al., 2003). The downregulation of Camkk2 observed in our study is consistent with its male-specific function in hippocampus memory formation and it may, at least partially underlie the mechanism of memory deficit in males after rmTBI.

Dlg4, also called PSD-95, interacts with the cytoplasmic tail of NMDA receptor subunits and shaker-type potassium channels and is believed to be required for synaptic plasticity associated with NMDA receptor signaling. Previous study revealed a significantly decreased level of PSD95 in the hippocampus from subjects with amnestic mild cognitive impairment, a prodromal stage of Alzheimer's disease (Sultana et al., 2010). A recent study also found downregulation of PSD-95 expression in the hippocampal synaptoproteome of aged rats compared with young and adult rats (VanGuilder et al., 2010). Thus altered synaptic protein PSD-95 expression after rmTBI may decrease stimulus-induced neurotransmission and vesicle replenishment and result in the following memory deficit.

Taken together, the current study find that repeated mild mechanical injury to the brain will result in changes of weekly BWGR, spatial learning and memory deficit, higher impulsivity in both male and female mice. Microarray analysis reveal that rmTBI pathophysiology is complex and involves altered expression levels of genes which could be broadly classified into a variety of molecular and cellular processes, including intracellular signaling, apoptosis and cell cycle, angiogenesis, cellular architecture, inflammation, oxidative stress, metabolism and transcriptional regulation and so on, all of which may contribute via multiple pathways to the post-traumatic sequelae seen in rmTBI. More specifically, genes involved in angiogenesis and inflammatory response tended to be enhanced in our study, while those related to energy metabolism, mitochondrial functions and synaptic plasticity were primarily depressed. The alterations

in those functional categories may reflect the general neuroprotective and repair mechanisms in response to the injury to facilitate cell survival and synaptic function or may be indicative of continuous damages as a result of the injury. It seems reasonable, then, to suggest that a therapeutic effect in rmTBI might be obtained by blocking acute secondary damage and limiting cell death. Although the alteration of some neuroprotective genes may be part of protective responses, the duration and/or magnitude of the endogenous alteration may be insufficient to prevent massive neuronal damage following rmTBI. Therefore, strategies to either increase the endogenous neuroprotective gene expression after rmTBI, or supplement it with exogenous ones, may improve neuronal survival and behavioral recovery after the injury.

Although there is no overlap of the differentially expressed genes between male and female mice, this should not be interpreted as sex-specific response, because the altered genes belong to the same functional categories. Further studies detecting gene expression changes at different time points following rmTBI in both sexes will provide a dynamic expression blueprint of those altered genes for better understanding the similar and differential response between male and female mice.

While the current study shows the advantage and convenience of microarray analysis in revealing multiple gene expression changes following rmTBI and serving as a baseline for evaluating the efficacy of future treatments, there are some difficulties with the use of microarray in studies of CNS. Firstly, the injury related changes of gene expression do not necessarily reflect the extent to which protein expression is affected,

the study dose not causally link these genes to pathogenesis following rmTBI. Secondly, it is hard to detect genes with low expression levels. It has been estimated that only 30% of the genes expressed within hippocampus are consistently detected on the Affymetrix GeneChip when using a gross dissection of the tissue (Evans et al., 2002). Thirdly, Since the changes in gene expression in CNS is always subtle while can be of major importance to altered brain function, using homogenates of the whole hippocampus might not be able to find the subtle, but important subregional changes in gene expression, especially of low-expressed genes. Furthermore, the changes in expression of some of these genes in our study do not concur with the direction previously reported, this is likely to be due to differences in experimental conditions (mouse model, severity of injury and time points after injury, etc.). Future studies using immunoblotting to confirm the directionality of protein of changes, proteomics-based analysis, pharmacological agents, cell-specific cultures and genetically manipulated animals to examine pathophysiology after the injury at different time points and in larger sample size will help address these questions. Nonetheless, the information we report here expands the breadth of gene expression changes after rmTBI, providing impetus for further investigation in the neuroprotective strategies following rmTBI.

Chapter 5: Conclusions

Proper CNS function depends on concerted expression of thousands of genes in a controlled and timely manner. We hypothesize that rmTBI results in neurological dysfunction, cognitive deficits and behavioral changes, which might be mediated by altered expression of some genes. Our data support the hypothesis by demonstrating significantly different changes in gene expression in rmTBI mice compared with sham-injury mice, besides transient neurological deficit, impaired weekly BWGR, changed behaviors in elevated plus maze, impaired spatial learning and memory in Morris water maze in the repeated mildly injured mice. The cellular functions in which the differentially expressed genes enriched include intracellular signaling, apoptosis and cell cycle, angiogenesis, cellular architecture, inflammation, oxidative stress, metabolism and transcriptional regulation and so on. RmTBI expression profiling revealed altered regulation of processes linked both to neural degeneration and regeneration. Our data did not specify whether the outcome of the altered responses in these functions are ultimately inhibited or stimulated. It is, however, not always possible to clearly differentiate genes contributing to neurodegenerative versus neuroprotective processes. Because of the important balance between neurodegenerative and regenerative processes following traumatic brain injury, future studies might profitably examine the contribution of genes identified here.

Given the complexity of the cellular responses to traumatic injury, longitudinal studies of responses are needed to fully identify and explore the resulting reparative and

degenerative processes since the functional deficits following rmTBI may derive from induction of neurotoxic factors that overwhelm endogenous neuroprotective responses.

Table 1. Classification of TBI based on injury severity

Criteria	Mild	Moderate	Severe
Structural imaging	Normal	Normal or abnormal	Normal or abnormal
Loss of Consciousness (LOC)	0-30 minutes	>30 min to <24 hours	> 24 hrs
Alteration of consciousness/mental state (AOC)	<24 hours	> 24 hrs	
Post-traumatic amnesia (PTA)	0–1 day	> 1 and < 7 days	> 7 days
Glascow Coma Scale (best available score in first 24 hours)	13-15	9-12	< 9

Table 2. rmTBI-induced gene expression changes in the hippocampus 4 weeks after injury according to the functional categories (fold change≥1.2) (female)

Gene Symbol	Illumina ID	Ratio※
1. Apoptosis & Cell cycle		
Axin2	ILMN_2896314	0.849605
Bcl11b	ILMN_2443624	1.313062
Bzw1	ILMN_2458986	1.292931
Coro1a	ILMN_2733793	1.261601
Crocc	ILMN_2616522	0.683923
Ddx5	ILMN_1215167	1.499846
Dido1	ILMN_3016099	0.611614
Dpysl5	ILMN_3161282	0.720616
Dusp8	ILMN_1228031	0.828714
E2f4	ILMN_2794796	0.649507
Hsf2	ILMN_2625520	1.582393
Lin54	ILMN_2793616	1.217224
Map3k12	ILMN_2725370	0.863358
Ndrg1	ILMN_1250195	0.808354
Nell2	ILMN_2711366	0.716759
Nfic	ILMN_1218384	0.785019
Pdcd4	ILMN_1253237	1.398464
Pdcd4	ILMN_2778122	1.425919
Polr2a	ILMN_2456696	0.602862
Ppm1l	ILMN_2419777	1.669212
Ptp4a2	ILMN_2611331	0.703495
Rpa1	ILMN_2750801	1.212272
Rsad1	ILMN_2802168	0.766051
Scrib	ILMN_2828731	0.677596
Sgpp1	ILMN_2877165	1.249968
Tgfbr1	ILMN_2602711	1.321255
Tia1	ILMN_2911936	0.788448
Txndc12	ILMN_1255473	0.761905
Zdhhc8	ILMN_2429203	0.747037
2. Metabolism (Ubiquitin-proteosome cascade and energy metabolism)		
Dgkz	ILMN_2915060	0.788089
Dgkz	ILMN_1239607	0.781519
Mat2a	ILMN_1258415	0.814795
Nhlrc1	ILMN_2595091	1.224507

Gene Symbol	Illumina ID	Ratio※
Ube2d2	ILMN_1215117	1.374088
Ube3b	ILMN_2855423	1.370576
Znrf1	ILMN_1232894	0.720808
Zubr1	ILMN_1222599	0.588736
Amy1	ILMN_2626453	0.686988
Coq3	ILMN_2973089	0.649216
mtDNA_ND5	ILMN_2507810	1.343626
Oat	ILMN_2933112	1.210212
Pde4b	ILMN_2544890	0.639919
Pex5	ILMN_3108328	0.788952
St8sia5	ILMN_1216035	0.774418
3. Inflammation		
AI851790	ILMN_2475795	1.222261
Btrc	ILMN_2690741	0.783826
Dab2	ILMN_1243329	1.519072
Dpp8	ILMN_1215849	1.440464
Fbxl11	ILMN_1224664	0.809442
Ikbkb	ILMN_2589556	0.692935
Irak1	ILMN_2518457	0.816343
Pou2f1	ILMN_2663366	0.789402
Sdc4	ILMN_2728729	1.300803
Sharpin	ILMN_1254001	0.805557
Usp52	ILMN_2790399	0.682577
4. Angiogenesis and endothelial function		
Bai2	ILMN_2813724	0.76791
Col4a1	ILMN_2621643	0.795611
Fhl2	ILMN_2770386	0.830366
Flt1	ILMN_1227926	0.696302
Mmp16	ILMN_2980226	1.368759
Ogn	ILMN_2859613	1.438084
Ptprb	ILMN_2591731	0.737044
5. Oxidative stress and NOS		
Atic	ILMN_3070951	0.670984
Dnajc18	ILMN_2615369	1.587429
Gstm2	ILMN_1251449	1.761748
Nme5	ILMN_2696232	1.279957
Nos3	ILMN_2788593	0.529374
Srxn1	ILMN_2667346	1.262081

Gene Symbol	Illumina ID	Ratio※
6. Cytoskeleton		
Actb	ILMN_2617433	1.569699
Actb	ILMN_1377923	1.587871
Ankrd47	ILMN_2963483	0.710503
Crocc	ILMN_2616522	0.683923
Frmd4a	ILMN_2429552	0.659788
Krt222	ILMN_2728021	0.635151
Parva	ILMN_1242472	1.389747
Ssh3	ILMN_1219080	0.576743
Tubb2b	ILMN_1221835	1.642949
Tubb2b	ILMN_2825574	1.597263
7. Transcriptional regulation, mRNA processing and protein level regulation		
Ahdc1	ILMN_2689187	0.765003
Ankrd13a	ILMN_2613696	0.814282
Arid1a	ILMN_1229829	0.650809
Atxn7l3	ILMN_1223340	0.702419
BC043301	ILMN_3114998	1.597227
Cic	ILMN_1216970	0.680428
Cnot3	ILMN_2987161	0.822698
Ldb2	ILMN_2754435	0.768394
Mbd1	ILMN_2893081	0.785339
Med24	ILMN_2818189	0.795315
Nacc1	ILMN_2842767	0.716603
Nfic	ILMN_1218384	0.785019
Pias3	ILMN_1227889	0.780546
Prmt6	ILMN_1221973	1.267863
Rtel1	ILMN_2641946	0.591409
Xrcc1	ILMN_3160475	0.830786
Zbtb38	ILMN_1250618	0.53219
Zfp655	ILMN_1219539	1.292337
Ars2	ILMN_1225615	0.712228
Elavl3	ILMN_2718837	0.804573
Hnrpdl	ILMN_2690061	0.789671
Nova2	ILMN_3044523	0.541945
Nudt21	ILMN_1234759	1.240928
Ptbp1	ILMN_3140788	0.795113
Sgpp1	ILMN_2877165	1.249968

Gene Symbol	Illumina ID	Ratio※
Ssu72	ILMN_1257298	1.216664
Syncrip	ILMN_1258420	1.236233
Furin	ILMN_3148489	0.804311
Inmt	ILMN_1231445	3.136847
Msl2	ILMN_2541591	1.723051
Wiz	ILMN_1221828	0.713457
8. Receptor and signal transduction		
Celsr3	ILMN_2604070	0.692209
Grik5	ILMN_2483633	0.745702
Igf1r	ILMN_1249824	0.767362
Phldb1	ILMN_2884126	0.726606
Pkd1	ILMN_2827036	0.65975
Fa2h	ILMN_2746783	0.800512
Gnal	ILMN_2429164	1.757524
Gpr155	ILMN_1230183	1.60361
Mpp3	ILMN_2698034	1.718946
Nisch	ILMN_1254218	0.731291
Rragb	ILMN_2923671	1.327035
9. Ion channel and transporter		
Nipa1	ILMN_2705349	1.540569
Slc17a7	ILMN_2618244	0.811335
Slc8a2	ILMN_2960263	0.716106
Tpcn1	ILMN_1217102	0.801436
10. Membrane protein and intracellular trafficking		
Ap2a1	ILMN_2604688	0.754333
Caln1	ILMN_1249022	0.827721
Cldn5	ILMN_1241293	0.626396
Coq3	ILMN_2973089	0.649216
Cpne8	ILMN_3151149	0.815203
Dctn4	ILMN_2712305	1.212141
Kifc2	ILMN_2695158	0.798979
Mapk8ip2	ILMN_2495555	1.240633
Rab3b	ILMN_1216073	1.51482
Rin2	ILMN_2663196	1.325944
Scrib	ILMN_2828731	0.677596
Snx16	ILMN_2736848	1.468293

Gene Symbol	Illumina ID	Ratio※
Spire1	ILMN_3115149	1.23273
Tram1	ILMN_2781938	1.252414
Wipi1	ILMN_1236411	0.61921
Wipi1	ILMN_2834677	0.709164
11. Synaptic function and plasticity		
Crtc1	ILMN_2680618	0.789176
Ctnnd2	ILMN_2634520	0.814015
Dgkb	ILMN_2442740	1.234496
Nrxn2	ILMN_2723541	0.634615

Ratio※: Fluorescence intensity relative to sham control (value of 1.0). Ratios from multiple hybridizations are present.

Table 3. rmTBI-induced gene expression changes in the hippocampus 4 weeks after injury according to the functional categories (fold change≥1.2) (male)

Gene Symbol	Illumina ID	Ratio※
1. Apoptosis & Cell cycle		
Cables1	ILMN_1217097	1.487305
Ccnt1	ILMN_2758087	0.774869
Prickle1	ILMN_1224069	0.748083
Sh2b1	ILMN_1256412	1.275731
Tnfrsf21	ILMN_2464573	0.670815
2. Metabolism (Ubiquitin-proteosome cascade and energy metabolism)		
Fbxo10	ILMN_2529395	1.207003
Stub1	ILMN_2628532	1.405201
4930438D12Rik	ILMN_2548916	0.658795
Coq7	ILMN_2729263	1.343595
Mrpl38	ILMN_2664049	1.276814
Sidt2	ILMN_1258738	0.772042
Slc25a38	ILMN_2946905	1.27076
3. Inflammation		
5730589K01Rik	ILMN_2779636	1.291694
Bat2	ILMN_1254154	0.5791
Errfi1	ILMN_2714031	1.264025
Neu1	ILMN_2708906	1.210429
5. Oxidative Stress		
Cyb5r1	ILMN_1243370	1.498097
Erp29	ILMN_1239185	1.236254
Gstz1	ILMN_1229964	1.284163
Mapk8ip1	ILMN_2871628	1.258849
6. Cytoskeleton		
LOC433749	ILMN_1219914	1.205532
Mtap6	ILMN_2439378	0.635724
Syne1	ILMN_1248614	0.687617
7. Transcriptional regulation, mRNA processing and protein level regulation		
Cited2	ILMN_2477221	1.238876
Gtf2h3	ILMN_1243127	1.243327
Hmg20a	ILMN_2968479	0.796552

Gene Symbol	Illumina ID	Ratio※
Lbh	ILMN_2816180	0.828177
Lbh	ILMN_1233545	0.597619
Lrpprc	ILMN_2683718	0.680503
Myst2	ILMN_2665161	0.751351
Polr1c	ILMN_1252845	1.307677
Rasl11a	ILMN_2932662	1.876731
Rreb1	ILMN_1255511	0.773705
5730406M06Rik	ILMN_1228707	0.655429
Dhx38	ILMN_3161767	1.209764
Hnrpl	ILMN_2627690	0.756669
Rnasen	ILMN_3144358	0.749248
4121402D02Rik	ILMN_1244356	0.747166
B3gat1	ILMN_2708717	0.818275
Eif3i	ILMN_2789601	1.285847
Extl3	ILMN_1239857	1.240578
Sult1a1	ILMN_2745370	1.570713
8. Receptor and signal transduction		
Gria2	ILMN_3122922	0.828443
Zranb1	ILMN_1249755	0.820862
9. Ion channel and transporter		
Kcna2	ILMN_2727857	0.737461
Kcnma1	ILMN_2723799	0.687341
Scn2a1	ILMN_1214686	0.809605
10. Membrane protein and intracellular trafficking		
Bzrap1	ILMN_1229256	0.791519
Cog8	ILMN_2595383	1.403383
Cope	ILMN_2944646	1.324935
Copz2	ILMN_2647028	1.398398
Cpne2	ILMN_2870487	1.578032
Gdi1	ILMN_2632299	0.824895
Gdi1	ILMN_2630975	0.768919
Rph3a	ILMN_2595600	0.736737
Ssr4	ILMN_1255237	1.216832
Surf4	ILMN_2595846	1.292928

Gene Symbol	Illumina ID	Ratio※
11. Synaptic function and plasticity		
Camkk2	ILMN_1256263	0.693758
Dlg4	ILMN_2710764	0.758182

Ratio※: Fluorescence intensity relative to sham control (value of 1.0). Ratios from multiple hybridizations are present.

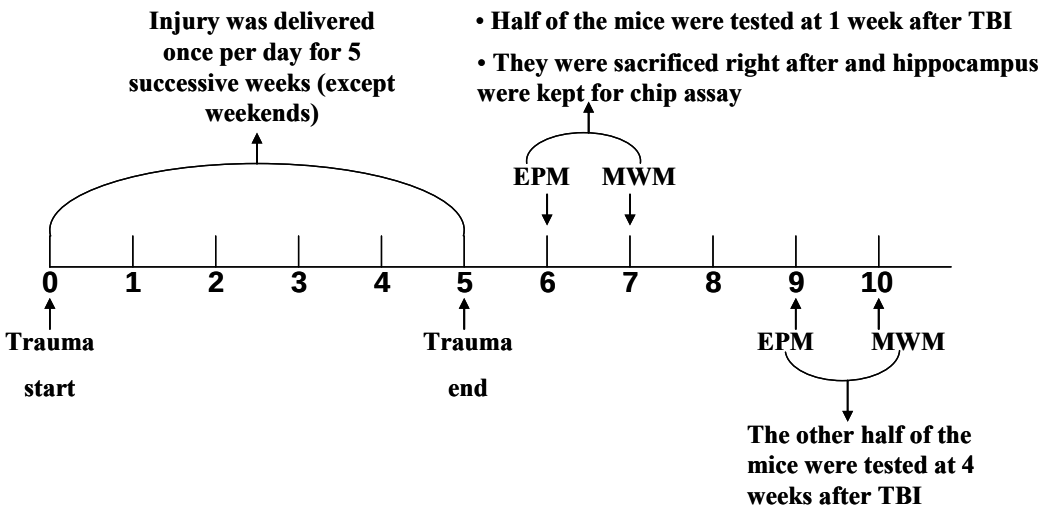


Figure 1. The time line of the whole experiment In both male and female groups, 10 mice were designated as control and 10 as TBI. All the TBI mice received the concussive-like head injury once a day for 5 successive weeks. After the 25 repeated head injuries, all the mice were divided into two batches with one batch tested at 1 week after the injury and another one tested at 4 weeks after the injury. The hippocampi from the mice tested at 1 week after rmTBI were kept for gene expression assay.

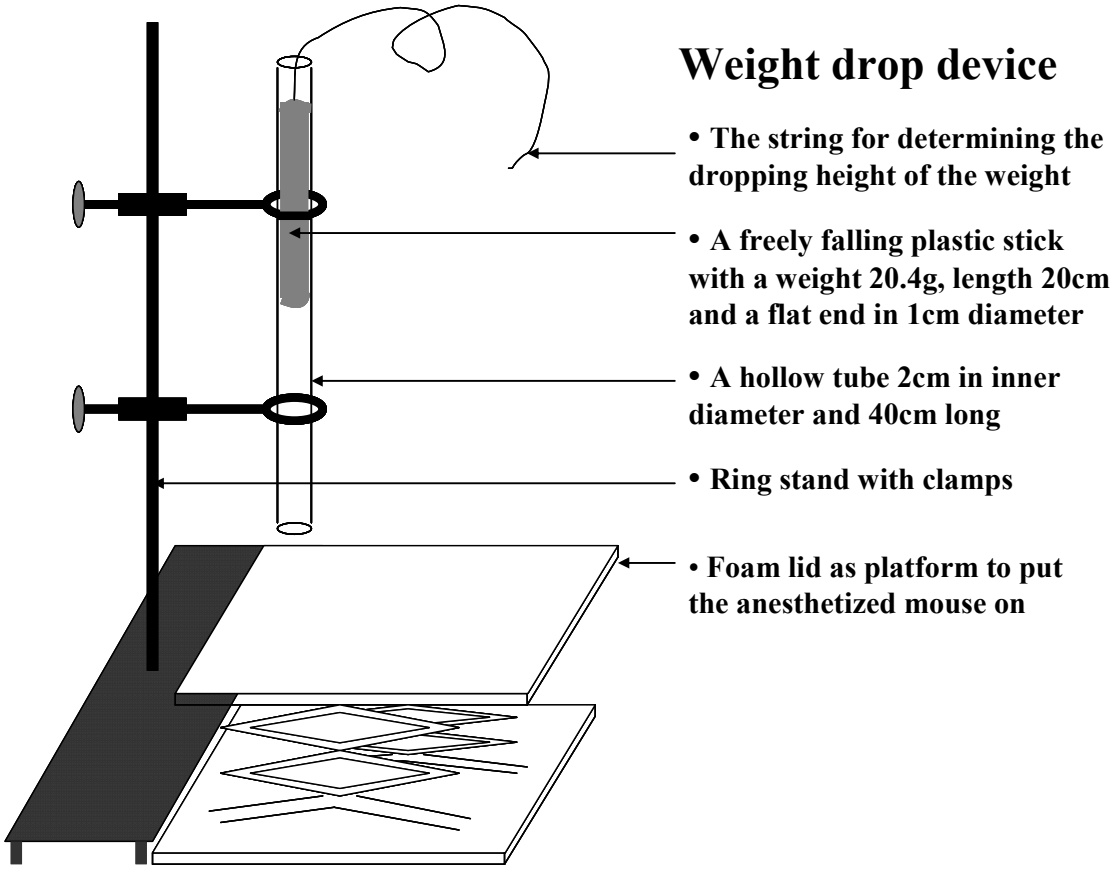


Figure 2. The weight drop device for producing rmTBI The weight drop device consists of a hollow Plexiglas tube, a cylindrical-shaped acrylic stick (20g) and a foam platform. The tube is kept vertical to the surface of the mouse head and guides the freely falling acrylic stick onto the head. The end of the stick is flat, round and 1cm in diameter which will hit the top of the mouse head encompassing the area over the frontal and parietal bones.

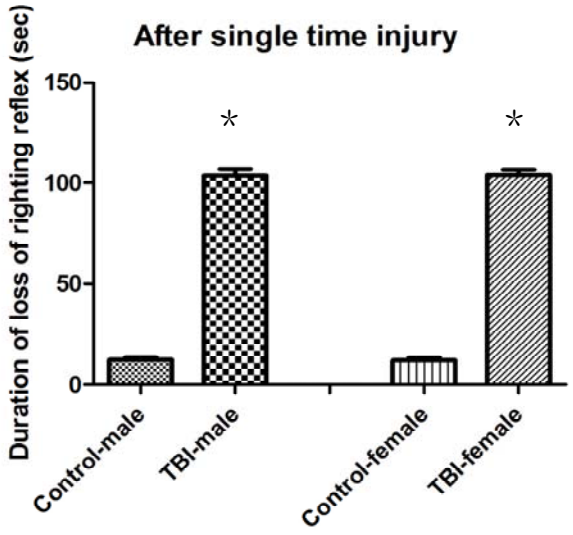
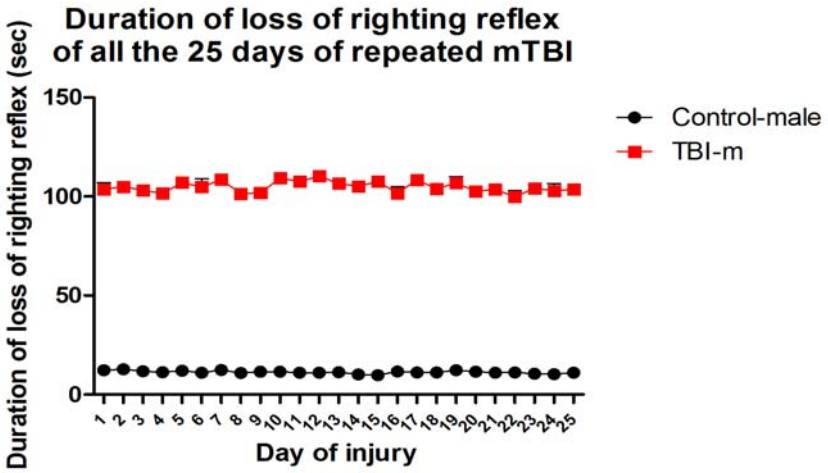
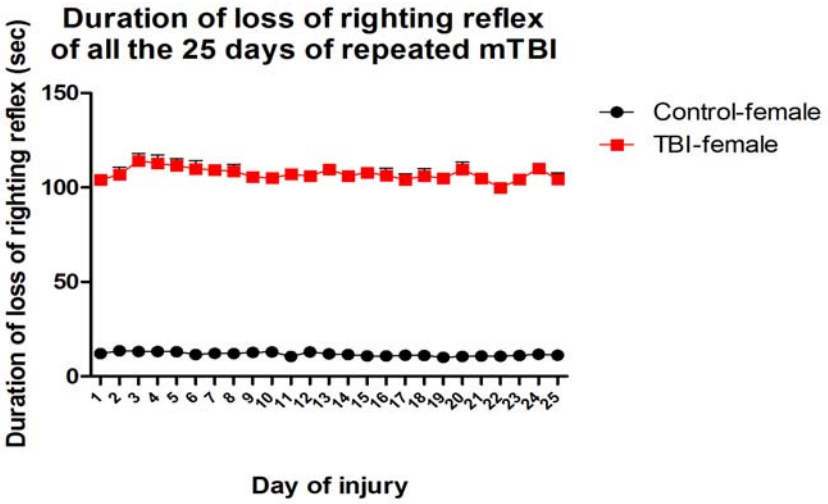


Figure 3. Duration of loss of righting reflex (sec) after single time injury After single time injury, the male TBI mice had an average duration of loss of righting reflex of 103.7±10.7 sec (mean±S.D.) and the female TBI mice had an average of 104±8.6 sec. While the control male and female mice had the average of 12.3±3.0 and 12.1±3.1 sec respectively. The result indicates that TBI mice had much longer duration of loss of conciousness after single time brain injury than control mice.



A



B

Figure 4. Duration of loss of righting reflex of all the 25 days of rmTBI The result showed that every day after the injury, there was a significant difference of duration of loss of righting reflex between TBI and their control in both sex. With the number of injuries increasing, the difference between TBI and control still exist in both male and female, but there was no significant increase or decrease of the duration within the male and female TBI mice respectively.

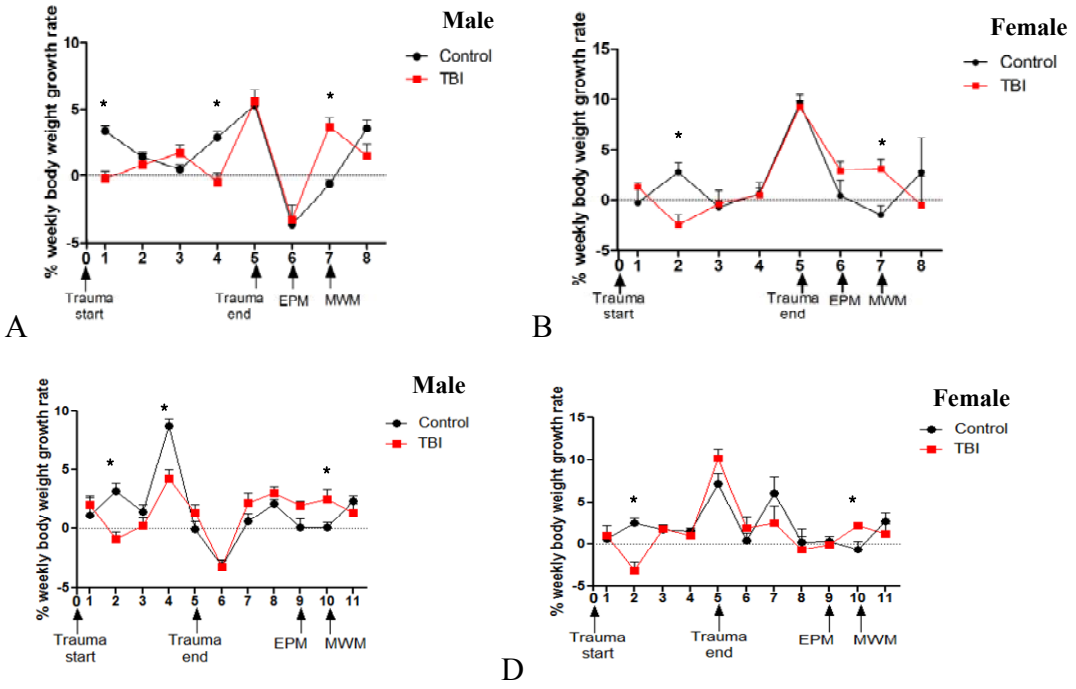
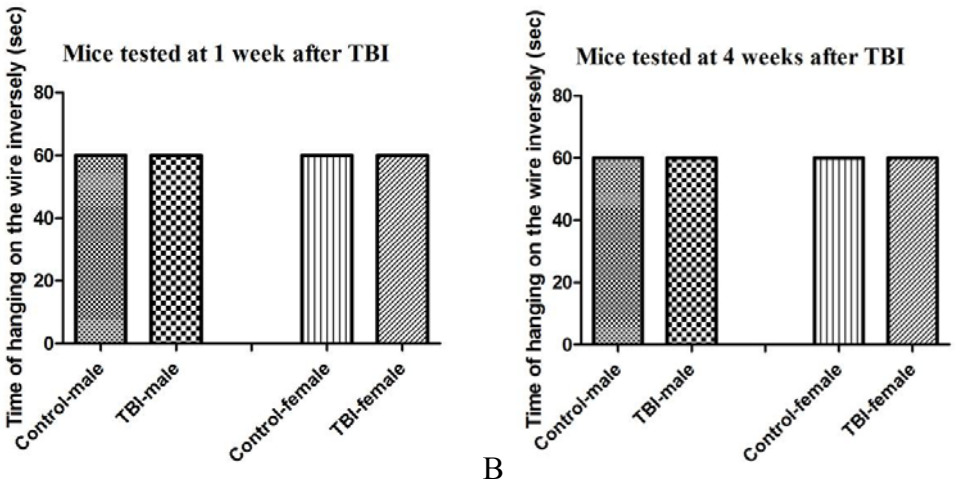
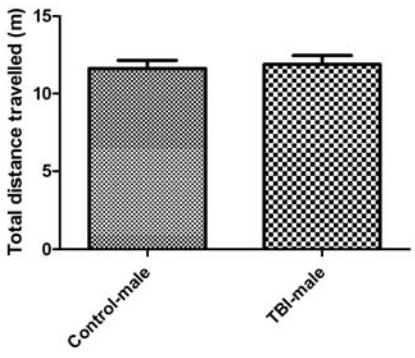


Figure 5. Weekly body weight growth rate during and after the rmTBI Fig. 5A) In the batch of mice tested at 1 week after rmTBI, male TBI mice had lower weekly BWGR than their control at the 1st and 4th week during the injury was applied daily. At the end of 5th week, when the injury ended, the TBI mice recovered to the same extent as the control. At the 7th week, when encountered the behavioral test (MWM), the weekly BWGR of the TBI mice become higher than that of the control ($P < 0.01$). And again, right after the stimulus of behavioral test, the TBI mice recovered to the same extent. Fig. 5B) Female mice tested at 1 week after rmTBI have a similar result of the male mice. Fig. 5C and 5D) In the batch of mice tested at 4 weeks after rmTBI, both the male and female TBI mice had transient lower weekly BWGR during the five weeks of injury application and these deficits recovered to the same level as the control by the end of the injury. When encountered environmental stimuli like behavioral tests, the TBI mice would possess a higher weekly BWGR than the control. This change of weekly BWGR will be gone after the finish of the stimuli.

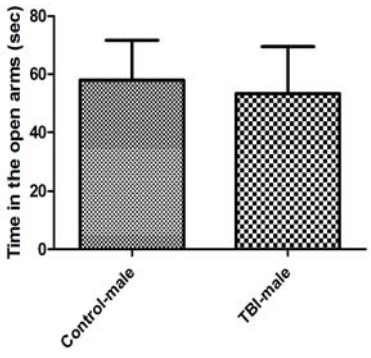


A B

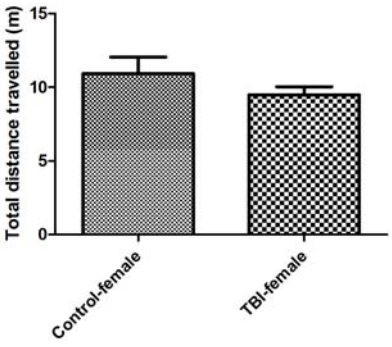
Figure 6. Motor function evaluation-wire hanging test At both 1week (Fig. 6A) and 4 weeks (Fig. 6B) after rmTBI, there was no difference of time hanging on the wire between TBI and their control in both male and female mice. All of them can hang on the wire for at least 60 sec, indicating that there was no motor dysfunction at those two time points after rmTBI.


Total distance travelled (m)
15
10
5
0
Control-male
TBI-male

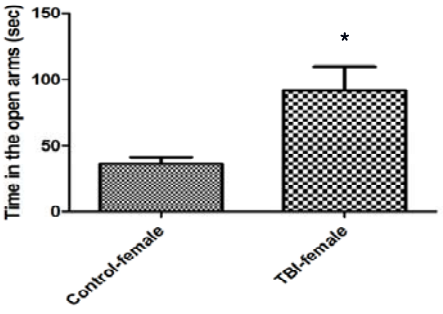
A


Time in the open arms (sec)
80
60
40
20
0
Control-male
TBI-male

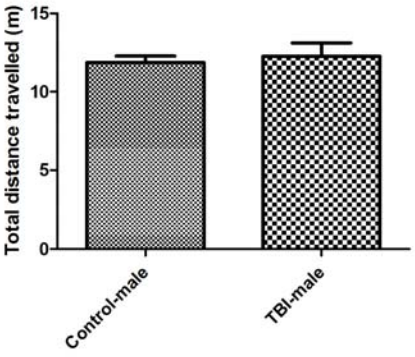
B


Total distance travelled (m)
15
10
5
0
Control-female
TBI-female

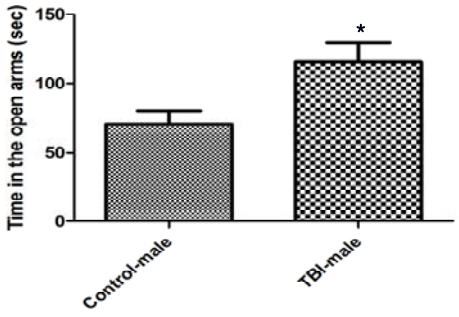
C


Time in the open arms (sec)
150
100
50
0
*
Control-female
TBI-female

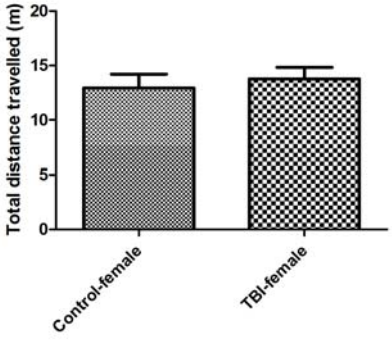
D


Total distance travelled (m)
15
10
5
0
Control-male
TBI-male

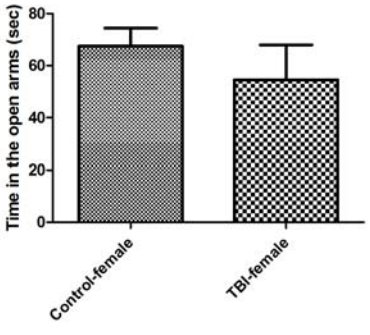
E


Time in the open arms (sec)
150
100
50
0
*
Control-male
TBI-male

F


Total distance travelled (m)
20
15
10
5
0
Control-female
TBI-female

G


Time in the open arms (sec)
80
60
40
20
0
Control-female
TBI-female

H

Figure 7. Elevated plus maze Fig. 7A, 7C, 7E and 7G) The total distance travelled in the maze of the TBI mice were not significantly different from the controls, in both male and female mice and at both 1 week and 4 weeks after the injury ($P > 0.05$). Fig. 7B) At 1 week after the injury, there was no significant difference of time spent in the open arms between male TBI mice and their control. Fig. 7D) At 1 week after the injury, female TBI mice spent much more time in the open arms than their control. Fig. 7F) At 4 weeks after the injury, the male TBI mice showed an increased time in the open arms. Fig. 7H) At 4 weeks after the injury, the difference of time spent in the open arms in the female group disappeared.

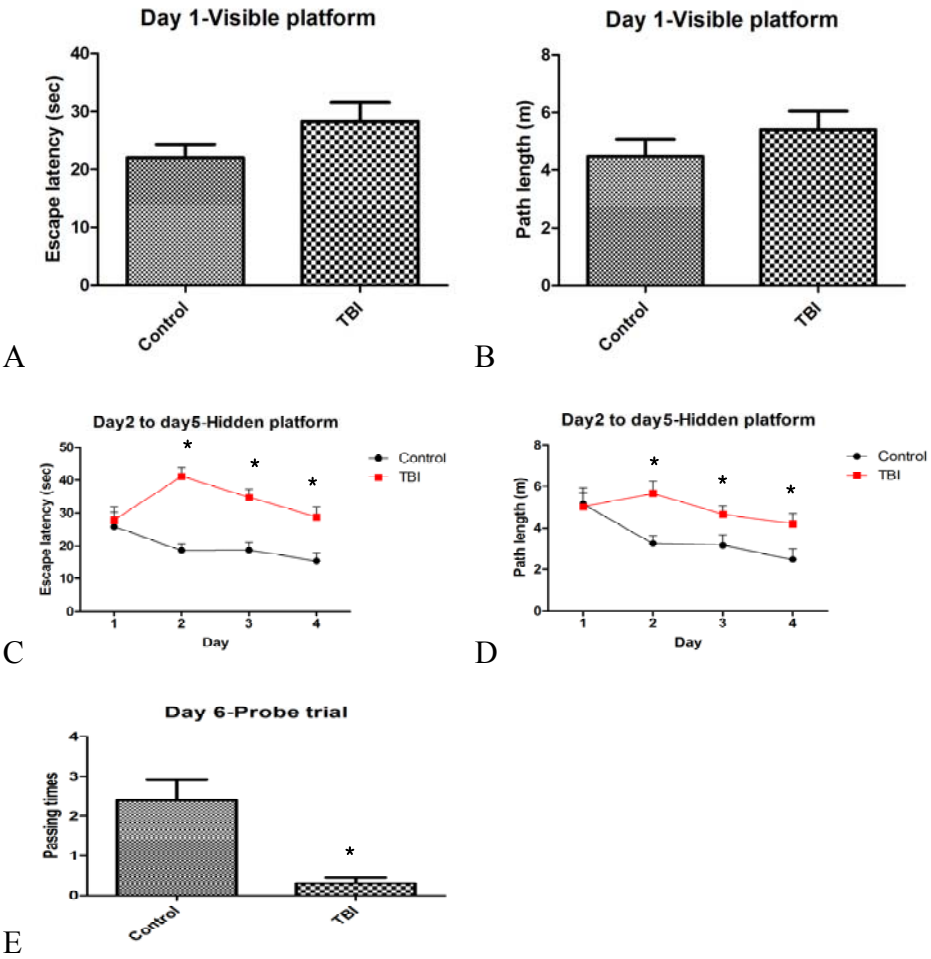


Figure 8. Morris water maze at 2weeks after injury Data of male and female mice were combined together in the analysis. At 2 weeks after the injury, Fig. 8A) in the visible platform tests, TBI and control mice had similar escape latency. Fig. 8B) TBI and control mice had similar path length. Fig. 8C) in the hidden platform test, TBI mice had longer escape latency on the 2nd, 3rd and 4th day of the hidden platform test than those of the control mice. Fig. 8D) TBI mice needed to swim significantly longer distances to reach the platform compared with control mice on the2nd, 3rd and 4th day. Fig. 8E) in the probe trial, the number of times the injured mice traveled into the platform zone, where the hidden platform was previously placed, was significantly less than that of the control.

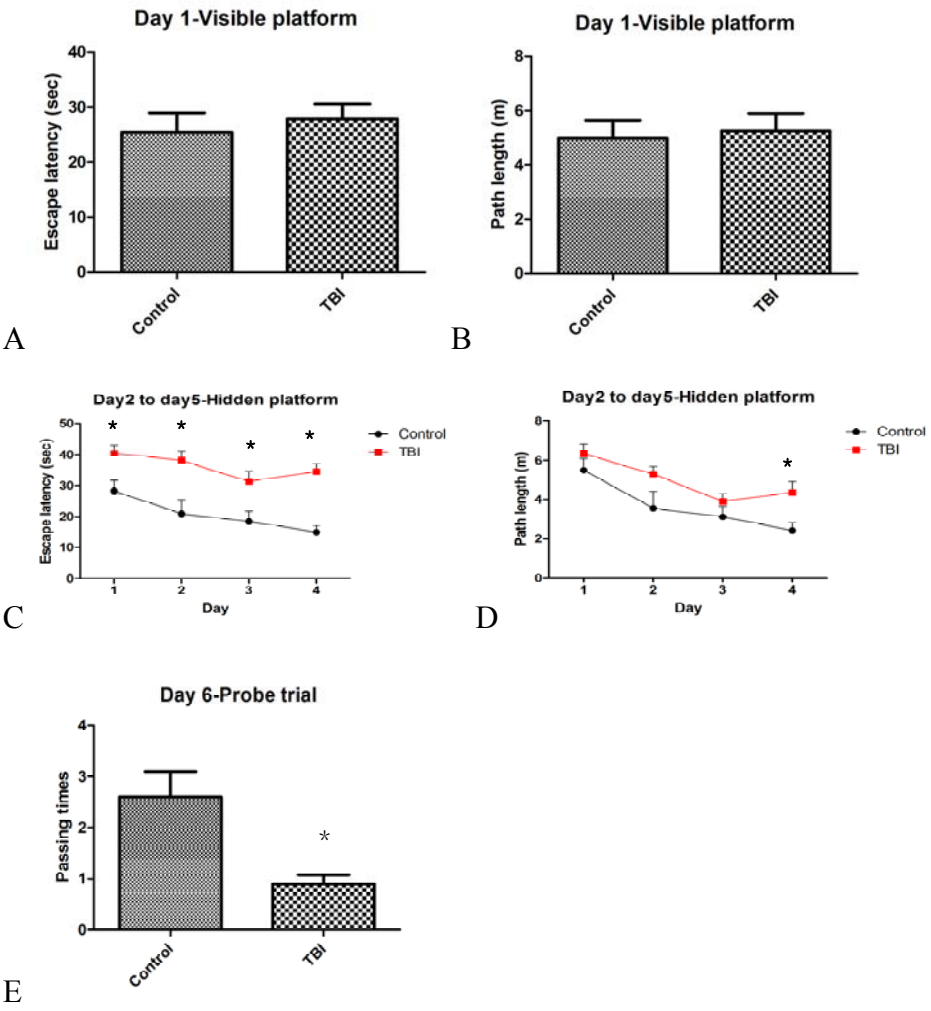


Figure 9. Morris water maze at 5 weeks after injury Data of male and female mice were combined together in the analysis. At 5 weeks after the injury, Fig. 9A and 9B) in the visible platform tests, there was no difference between TBI and control in escape latency and path length. Fig. 9C) in the hidden platform-swimming test, the escape latency on all the four days of TBI mice were longer than those of the control mice. Fig. 9D) TBI mice needed to swim significantly longer distances to reach the platform compared with control mice on the fourth day. Fig. 9E) in the probe trial, TBI mice had less numbers of entries into the platform zone compared with control.

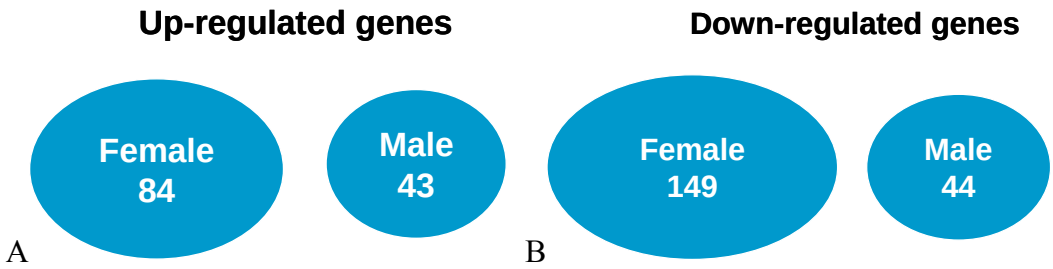


Figure 10. Number of genes differentially expressed after rmTBI revealed by Illumina whole genome gene expression assay In male TBI mice, a total of 87 genes were identified to be differentially expressed, and in female TBI mice, 233 genes were differentially expressed after rmTBI. Among the altered genes, 43 were up-regulated and 44 were down-regulated in male TBI mice while in female TBI mice, 84 were up-regulated and 149 were down-regulated.

References

Adekoya, N., Thurman, D.J., White, D.D., and Webb, K.W. (2002). Surveillance for traumatic brain injury deaths--United States, 1989-1998. MMWR Surveill Summ *51*, 1-14.

Albensi, B.C. (2001). Models of brain injury and alterations in synaptic plasticity. J Neurosci Res *65*, 279-283.

Annegers, J.F., and Coan, S.P. (2000). The risks of epilepsy after traumatic brain injury. Seizure *9*, 453-457.

Araki, T., and Milbrandt, J. (2003). ZNRF proteins constitute a family of presynaptic E3 ubiquitin ligases. J Neurosci *23*, 9385-9394.

Autti, T., Sipila, L., Autti, H., and Salonen, O. (1997). Brain lesions in players of contact sports. Lancet *349*, 1144.

Ayasolla, K.R., Singh, A.K., and Singh, I. (2005). 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranoside (AICAR) attenuates the expression of LPS- and Abeta peptide-induced inflammatory mediators in astroglia. J Neuroinflammation *2*, 21.

Babbs, C.F. (2000). Brain injury in amateur soccer players. JAMA *283*, 882; author reply 883.

Bahe, S., Stierhof, Y.D., Wilkinson, C.J., Leiss, F., and Nigg, E.A. (2005). Rootletin forms centriole-associated filaments and functions in centrosome cohesion. J Cell Biol *171*, 27-33.

Bannai, H., Fukatsu, K., Mizutani, A., Natsume, T., Iemura, S., Ikegami, T., Inoue, T., and Mikoshiba, K. (2004). An RNA-interacting protein, SYNCRIP (heterogeneous nuclear ribonuclear protein Q1/NSAP1) is a component of mRNA granule transported with inositol 1,4,5-trisphosphate receptor type 1 mRNA in neuronal dendrites. J Biol Chem *279*, 53427-53434.

Beeler, N., Riederer, B.M., Waeber, G., and Abderrahmani, A. (2009). Role of the JNK-interacting protein 1/islet brain 1 in cell degeneration in Alzheimer disease and diabetes. Brain Res Bull *80*, 274-281.

Bennett, B.D., Denis, P., Haniu, M., Teplow, D.B., Kahn, S., Louis, J.C., Citron, M., and

Vassar, R. (2000). A furin-like convertase mediates propeptide cleavage of BACE, the Alzheimer's beta -secretase. J Biol Chem *275*, 37712-37717.

Bergstedt, K., and Wieloch, T. (1993). Changes in insulin-like growth factor 1 receptor density after transient cerebral ischemia in the rat. Lack of protection against ischemic brain damage following injection of insulin-like growth factor 1. J Cereb Blood Flow Metab *13*, 895-898.

Bernert, H., and Turski, L. (1996). Traumatic brain damage prevented by the non-N-methyl-D-aspartate antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f] quinoxaline. Proc Natl Acad Sci U S A *93*, 5235-5240.

Bethea, J.R. (2000). Spinal cord injury-induced inflammation: a dual-edged sword. Prog Brain Res *128*, 33-42.

Bliss, T.V., and Collingridge, G.L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. Nature *361*, 31-39.

Brochier, C., Gaillard, M.C., Diguet, E., Caudy, N., Dossat, C., Segurens, B., Wincker, P., Roze, E., Caboche, J., Hantraye, P.*, et al.* (2008). Quantitative gene expression profiling of mouse brain regions reveals differential transcripts conserved in human and affected in disease models. Physiol Genomics *33*, 170-179.

Broer, A., Wagner, C.A., Lang, F., and Broer, S. (2000). The heterodimeric amino acid transporter 4F2hc/y+LAT2 mediates arginine efflux in exchange with glutamine. Biochem J *349 Pt 3*, 787-795.

Cantu, R.C. (2007). Chronic traumatic encephalopathy in the National Football League. Neurosurgery *61*, 223-225.

Cassidy, J.D., Carroll, L.J., Peloso, P.M., Borg, J., von Holst, H., Holm, L., Kraus, J., and Coronado, V.G. (2004). Incidence, risk factors and prevention of mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J Rehabil Med, 28-60.

Chanas, S.A., Jiang, Q., McMahon, M., McWalter, G.K., McLellan, L.I., Elcombe, C.R., Henderson, C.J., Wolf, C.R., Moffat, G.J., Itoh, K.*, et al.* (2002). Loss of the Nrf2 transcription factor causes a marked reduction in constitutive and inducible expression of the glutathione S-transferase Gsta1, Gsta2, Gstm1, Gstm2, Gstm3 and Gstm4 genes in the livers of male and female mice. Biochem J *365*, 405-416.

Cheng, K.K., Lam, K.S., Wang, Y., Huang, Y., Carling, D., Wu, D., Wong, C., and Xu, A. (2007). Adiponectin-induced endothelial nitric oxide synthase activation and nitric oxide production are mediated by APPL1 in endothelial cells. Diabetes *56*, 1387-1394.

Cherry, J.A., and Davis, R.L. (1999). Cyclic AMP phosphodiesterases are localized in regions of the mouse brain associated with reinforcement, movement, and affect. J Comp Neurol *407*, 287-301.

Colicos, M.A., and Dash, P.K. (1996). Apoptotic morphology of dentate gyrus granule cells following experimental cortical impact injury in rats: possible role in spatial memory deficits. Brain Res *739*, 120-131.

Collins, M.W., Grindel, S.H., Lovell, M.R., Dede, D.E., Moser, D.J., Phalin, B.R., Nogle, S., Wasik, M., Cordry, D., Daugherty, K.M.*, et al.* (1999). Relationship between concussion and neuropsychological performance in college football players. JAMA *282*, 964-970.

Conti, B., Maier, R., Barr, A.M., Morale, M.C., Lu, X., Sanna, P.P., Bilbe, G., Hoyer, D., and Bartfai, T. (2007). Region-specific transcriptional changes following the three antidepressant treatments electro convulsive therapy, sleep deprivation and fluoxetine. Mol Psychiatry *12*, 167-189.

Cottrell, J.R., Borok, E., Horvath, T.L., and Nedivi, E. (2004). CPG2: a brain- and synapse-specific protein that regulates the endocytosis of glutamate receptors. Neuron *44*, 677-690.

Creeley, C.E., Wozniak, D.F., Bayly, P.V., Olney, J.W., and Lewis, L.M. (2004). Multiple episodes of mild traumatic brain injury result in impaired cognitive performance in mice. Acad Emerg Med *11*, 809-819.

D'Ambrosio, R., and Perucca, E. (2004). Epilepsy after head injury. Curr Opin Neurol *17*, 731-735.

D'Ercole, A.J., Ye, P., Calikoglu, A.S., and Gutierrez-Ospina, G. (1996). The role of the insulin-like growth factors in the central nervous system. Mol Neurobiol *13*, 227-255.

Dash, P.K., Mach, S.A., and Moore, A.N. (2001). Enhanced neurogenesis in the rodent hippocampus following traumatic brain injury. J Neurosci Res *63*, 313-319.

DeFord, S.M., Wilson, M.S., Rice, A.C., Clausen, T., Rice, L.K., Barabnova, A., Bullock, R., and Hamm, R.J. (2002). Repeated mild brain injuries result in cognitive impairment

in B6C3F1 mice. J Neurotrauma *19*, 427-438.

Deitmer, J.W., Broer, A., and Broer, S. (2003). Glutamine efflux from astrocytes is mediated by multiple pathways. J Neurochem *87*, 127-135.

Di Giovanni, S., Movsesyan, V., Ahmed, F., Cernak, I., Schinelli, S., Stoica, B., and Faden, A.I. (2005). Cell cycle inhibition provides neuroprotection and reduces glial proliferation and scar formation after traumatic brain injury. Proc Natl Acad Sci U S A *102*, 8333-8338.

Dixon, C.E., Clifton, G.L., Lighthall, J.W., Yaghmai, A.A., and Hayes, R.L. (1991). A controlled cortical impact model of traumatic brain injury in the rat. J Neurosci Methods *39*, 253-262.

Dodson, G.E., Shi, Y., and Tibbetts, R.S. (2004). DNA replication defects, spontaneous DNA damage, and ATM-dependent checkpoint activation in replication protein A-deficient cells. J Biol Chem *279*, 34010-34014.

Donnelly, D.J., and Popovich, P.G. (2008). Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. Exp Neurol *209*, 378-388.

Earls, L.R., Hacker, M.L., Watson, J.D., and Miller, D.M., 3rd (2010). Coenzyme Q protects Caenorhabditis elegans GABA neurons from calcium-dependent degeneration. Proc Natl Acad Sci U S A *107*, 14460-14465.

Eastwood, S.L., Lyon, L., George, L., Andrieux, A., Job, D., and Harrison, P.J. (2007). Altered expression of synaptic protein mRNAs in STOP (MAP6) mutant mice. J Psychopharmacol *21*, 635-644.

Ellis, E.F., McKinney, J.S., Willoughby, K.A., Liang, S., and Povlishock, J.T. (1995). A new model for rapid stretch-induced injury of cells in culture: characterization of the model using astrocytes. J Neurotrauma *12*, 325-339.

Espana, J., Valero, J., Minano-Molina, A.J., Masgrau, R., Martin, E., Guardia-Laguarta, C., Lleo, A., Gimenez-Llort, L., Rodriguez-Alvarez, J., and Saura, C.A. (2010). beta-Amyloid disrupts activity-dependent gene transcription required for memory through the CREB coactivator CRTC1. J Neurosci *30*, 9402-9410.

Evans, S.J., Datson, N.A., Kabbaj, M., Thompson, R.C., Vreugdenhil, E., De Kloet, E.R., Watson, S.J., and Akil, H. (2002). Evaluation of Affymetrix Gene Chip sensitivity in rat

hippocampal tissue using SAGE analysis. Serial Analysis of Gene Expression. Eur J Neurosci *16*, 409-413.

Faul M, Xu L, Wald MM, and VG., C. (2010). Traumatic brain injury in the United States: emergency department visits, hospitalizations, and deaths. 2002-2006 (Atlanta (GA), Centers for Disease Control and Prevention, National Center for Injury Prevention and Control).

Ferko, A.P. (1986). Effects of L-ascorbic acid on ethanol-induced central nervous system depression in mice. Pharmacol Biochem Behav *24*, 543-547.

Fiorentino, L., Stehlik, C., Oliveira, V., Ariza, M.E., Godzik, A., and Reed, J.C. (2002). A novel PAAD-containing protein that modulates NF-kappa B induction by cytokines tumor necrosis factor-alpha and interleukin-1beta. J Biol Chem *277*, 35333-35340.

Fox, G.B., LeVasseur, R.A., and Faden, A.I. (1999). Behavioral responses of C57BL/6, FVB/N, and 129/SvEMS mouse strains to traumatic brain injury: implications for gene targeting approaches to neurotrauma. J Neurotrauma *16*, 377-389.

Freeman, R.S., Estus, S., and Johnson, E.M., Jr. (1994). Analysis of cell cycle-related gene expression in postmitotic neurons: selective induction of Cyclin D1 during programmed cell death. Neuron *12*, 343-355.

Fu, Q.Y., and Gao, Y.Q. (2009). Screening of AMP-activated protein kinase alpha2 subunit interacting proteins by bacterial two-hybrid system. Mol Biol Rep *36*, 337-344.

Gaetz, M., Goodman, D., and Weinberg, H. (2000). Electrophysiological evidence for the cumulative effects of concussion. Brain Inj *14*, 1077-1088.

Garcia-Domingo, D., Leonardo, E., Grandien, A., Martinez, P., Albar, J.P., Izpisua-Belmonte, J.C., and Martinez, A.C. (1999). DIO-1 is a gene involved in onset of apoptosis in vitro, whose misexpression disrupts limb development. Proc Natl Acad Sci U S A *96*, 7992-7997.

Garyali, P., Siwach, P., Singh, P.K., Puri, R., Mittal, S., Sengupta, S., Parihar, R., and Ganesh, S. (2009). The malin-laforin complex suppresses the cellular toxicity of misfolded proteins by promoting their degradation through the ubiquitin-proteasome system. Hum Mol Genet *18*, 688-700.

Gavarini, S., Becamel, C., Altier, C., Lory, P., Poncet, J., Wijnholds, J., Bockaert, J., and Marin, P. (2006). Opposite effects of PSD-95 and MPP3 PDZ proteins on serotonin

5-hydroxytryptamine2C receptor desensitization and membrane stability. Mol Biol Cell *17*, 4619-4631.

Gogolla, N., Galimberti, I., Deguchi, Y., and Caroni, P. (2009). Wnt signaling mediates experience-related regulation of synapse numbers and mossy fiber connectivities in the adult hippocampus. Neuron *62*, 510-525.

Gohil, V.M., Nilsson, R., Belcher-Timme, C.A., Luo, B., Root, D.E., and Mootha, V.K. (2010). Mitochondrial and nuclear genomic responses to loss of LRPPRC expression. J Biol Chem *285*, 13742-13747.

Gonzalez, Y.R., Zhang, Y., Behzadpoor, D., Cregan, S., Bamforth, S., Slack, R.S., and Park, D.S. (2008). CITED2 signals through peroxisome proliferator-activated receptor-gamma to regulate death of cortical neurons after DNA damage. J Neurosci *28*, 5559-5569.

Goodman, J.C., Cherian, L., Bryan, R.M., Jr., and Robertson, C.S. (1994). Lateral cortical impact injury in rats: pathologic effects of varying cortical compression and impact velocity. J Neurotrauma *11*, 587-597.

Graham DI, Gennarelli TA, and 830–835., M.T. (2002). Greenfield's Neuropathology. , 7th edn (Arnold; London).

Gray, J., and McNaughton, N. (2000). The Neuropsychology of Anxiety: An Enquiry into the Funtions of the Septo-Hippocampal System (Oxford University Press).

Hanlon, D.W., Yang, Z., and Goldstein, L.S. (1997). Characterization of KIFC2, a neuronal kinesin superfamily member in mouse. Neuron *18*, 439-451.

Harmon, K.G. (1999). Assessment and management of concussion in sports. Am Fam Physician *60*, 887-892, 894.

Hodges, A., Strand, A.D., Aragaki, A.K., Kuhn, A., Sengstag, T., Hughes, G., Elliston, L.A., Hartog, C., Goldstein, D.R., Thu, D., *et al.* (2006). Regional and cellular gene expression changes in human Huntington's disease brain. Hum Mol Genet *15*, 965-977.

Hofman, P.A., Stapert, S.Z., van Kroonenburgh, M.J., Jolles, J., de Kruijk, J., and Wilmink, J.T. (2001). MR imaging, single-photon emission CT, and neurocognitive performance after mild traumatic brain injury. AJNR Am J Neuroradiol *22*, 441-449.

Holm, L., Cassidy, J.D., Carroll, L.J., and Borg, J. (2005). Summary of the WHO

Collaborating Centre for Neurotrauma Task Force on Mild Traumatic Brain Injury. J Rehabil Med *37*, 137-141.

Huang, C.S., Shi, S.H., Ule, J., Ruggiu, M., Barker, L.A., Darnell, R.B., Jan, Y.N., and Jan, L.Y. (2005). Common molecular pathways mediate long-term potentiation of synaptic excitation and slow synaptic inhibition. Cell *123*, 105-118.

Iizuka, M., Sarmento, O.F., Sekiya, T., Scrable, H., Allis, C.D., and Smith, M.M. (2008). Hbo1 Links p53-dependent stress signaling to DNA replication licensing. Mol Cell Biol *28*, 140-153.

Ikenouchi, J., and Umeda, M. (2010). FRMD4A regulates epithelial polarity by connecting Arf6 activation with the PAR complex. Proc Natl Acad Sci U S A *107*, 748-753.

Ikonomidou, C., and Turski, L. (1996). Prevention of trauma-induced neurodegeneration in infant and adult rat brain: glutamate antagonists. Metab Brain Dis *11*, 125-141.

Iseki, K., Hagino, S., Mori, T., Zhang, Y., Yokoya, S., Takaki, H., Tase, C., Murakawa, M., and Wanaka, A. (2002). Increased syndecan expression by pleiotrophin and FGF receptor-expressing astrocytes in injured brain tissue. Glia *39*, 1-9.

Ishizaki, H., Miyoshi, J., Kamiya, H., Togawa, A., Tanaka, M., Sasaki, T., Endo, K., Mizoguchi, A., Ozawa, S., and Takai, Y. (2000). Role of rab GDP dissociation inhibitor alpha in regulating plasticity of hippocampal neurotransmission. Proc Natl Acad Sci U S A *97*, 11587-11592.

Jaglin, X.H., and Chelly, J. (2009). Tubulin-related cortical dysgeneses: microtubule dysfunction underlying neuronal migration defects. Trends Genet *25*, 555-566.

Jana, A., Hogan, E.L., and Pahan, K. (2009). Ceramide and neurodegeneration: susceptibility of neurons and oligodendrocytes to cell damage and death. J Neurol Sci *278*, 5-15.

Javelaud, D., and Besancon, F. (2001). NF-kappa B activation results in rapid inactivation of JNK in TNF alpha-treated Ewing sarcoma cells: a mechanism for the anti-apoptotic effect of NF-kappa B. Oncogene *20*, 4365-4372.

JE., B. (1998). Head injuries in sports., 2nd edn (Philadelphia, Lippincott- Raven).

Jelen, N., Ule, J., and Zivin, M. (2010). Cholinergic regulation of striatal Nova mRNAs.

Neuroscience *169*, 619-627.

Jennett, B. (1998). Epidemiology of head injury. Arch Dis Child *78*, 403-406.

Jeong, W., Lee, D.Y., Park, S., and Rhee, S.G. (2008). ERp16, an endoplasmic reticulum-resident thiol-disulfide oxidoreductase: biochemical properties and role in apoptosis induced by endoplasmic reticulum stress. J Biol Chem *283*, 25557-25566.

Jiang, H.X., Guan, Q.H., Pei, D.S., and Zhang, G.Y. (2007). Functional cooperation between KA2 and GluR6 subunits is involved in the ischemic brain injury. J Neurosci Res *85*, 2960-2970.

Jin, G., Arai, K., Murata, Y., Wang, S., Stins, M.F., Lo, E.H., and van Leyen, K. (2008). Protecting against cerebrovascular injury: contributions of 12/15-lipoxygenase to edema formation after transient focal ischemia. Stroke *39*, 2538-2543.

Jones, S.L., Wang, J., Turck, C.W., and Brown, E.J. (1998). A role for the actin-bundling protein L-plastin in the regulation of leukocyte integrin function. Proc Natl Acad Sci U S A *95*, 9331-9336.

Joseph, P., Lei, Y.X., and Ong, T.M. (2004). Up-regulation of expression of translation factors--a novel molecular mechanism for cadmium carcinogenesis. Mol Cell Biochem *255*, 93-101.

Kanazawa, S., Soucek, L., Evan, G., Okamoto, T., and Peterlin, B.M. (2003). c-Myc recruits P-TEFb for transcription, cellular proliferation and apoptosis. Oncogene *22*, 5707-5711.

Kelley, R.L., Solovyeva, I., Lyman, L.M., Richman, R., Solovyev, V., and Kuroda, M.I. (1995). Expression of msl-2 causes assembly of dosage compensation regulators on the X chromosomes and female lethality in Drosophila. Cell *81*, 867-877.

Kelly, J.P., and Rosenberg, J.H. (1997). Diagnosis and management of concussion in sports. Neurology *48*, 575-580.

Klein, J.A., and Ackerman, S.L. (2003). Oxidative stress, cell cycle, and neurodegeneration. J Clin Invest *111*, 785-793.

Kraus, J.F., and McArthur, D.L. (1996). Epidemiologic aspects of brain injury. Neurol Clin *14*, 435-450.

Krum, J.M., Mani, N., and Rosenstein, J.M. (2008). Roles of the endogenous VEGF receptors flt-1 and flk-1 in astroglial and vascular remodeling after brain injury. Exp Neurol *212*, 108-117.

Kruse, J.P., and Gu, W. (2009). MSL2 promotes Mdm2-independent cytoplasmic localization of p53. J Biol Chem *284*, 3250-3263.

Lachat, P., Shaw, P., Gebhard, S., van Belzen, N., Chaubert, P., and Bosman, F.T. (2002). Expression of NDRG1, a differentiation-related gene, in human tissues. Histochem Cell Biol *118*, 399-408.

Lafleur, M.A., Handsley, M.M., Knauper, V., Murphy, G., and Edwards, D.R. (2002). Endothelial tubulogenesis within fibrin gels specifically requires the activity of membrane-type-matrix metalloproteinases (MT-MMPs). J Cell Sci *115*, 3427-3438.

Laurer, H.L., Bareyre, F.M., Lee, V.M., Trojanowski, J.Q., Longhi, L., Hoover, R., Saatman, K.E., Raghupathi, R., Hoshino, S., Grady, M.S., *et al.* (2001). Mild head injury increasing the brain's vulnerability to a second concussive impact. J Neurosurg *95*, 859-870.

Lee, J.H., Cheng, R., Barral, S., Reitz, C., Medrano, M., Lantigua, R., Jimenez-Velazquez, I.Z., Rogaeva, E., St George-Hyslop, P.H., and Mayeux, R. (2010). Identification of Novel Loci for Alzheimer Disease and Replication of CLU, PICALM, and BIN1 in Caribbean Hispanic Individuals. Arch Neurol.

Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., Yim, J., Lee, J., Provost, P., Radmark, O., Kim, S., *et al.* (2003). The nuclear RNase III Drosha initiates microRNA processing. Nature *425*, 415-419.

Li, N., Ragheb, K., Lawler, G., Sturgis, J., Rajwa, B., Melendez, J.A., and Robinson, J.P. (2003). Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. J Biol Chem *278*, 8516-8525.

Lighthall, J.W. (1988). Controlled cortical impact: a new experimental brain injury model. J Neurotrauma *5*, 1-15.

Lin, C.L., Bristol, L.A., Jin, L., Dykes-Hoberg, M., Crawford, T., Clawson, L., and Rothstein, J.D. (1998). Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. Neuron *20*, 589-602.

Lin, D.C., Quevedo, C., Brewer, N.E., Bell, A., Testa, J.R., Grimes, M.L., Miller, F.D., and Kaplan, D.R. (2006). APPL1 associates with TrkA and GIPC1 and is required for nerve growth factor-mediated signal transduction. Mol Cell Biol *26*, 8928-8941.

Liu, X., Cheng, Y., Yang, J., Krall, T.J., Huo, Y., and Zhang, C. (2010). An essential role of PDCD4 in vascular smooth muscle cell apoptosis and proliferation: implications for vascular disease. Am J Physiol Cell Physiol *298*, C1481-1488.

Longhi, L., Saatman, K.E., Fujimoto, S., Raghupathi, R., Meaney, D.F., Davis, J., McMillan, B.S.A., Conte, V., Laurer, H.L., Stein, S., *et al.* (2005). Temporal window of vulnerability to repetitive experimental concussive brain injury. Neurosurgery *56*, 364-374; discussion 364-374.

Love, S. (1999). Oxidative stress in brain ischemia. Brain Pathol *9*, 119-131.

Lu, T., Jackson, M.W., Wang, B., Yang, M., Chance, M.R., Miyagi, M., Gudkov, A.V., and Stark, G.R. (2010a). Regulation of NF-kappaB by NSD1/FBXL11-dependent reversible lysine methylation of p65. Proc Natl Acad Sci U S A *107*, 46-51.

Lu, W.C., Chen, C.J., Hsu, H.C., Hsu, H.L., and Chen, L. (2010b). The adaptor protein SH2B1beta reduces hydrogen peroxide-induced cell death in PC12 cells and hippocampal neurons. J Mol Signal *5*, 17.

Madathil, S.K., Evans, H.N., and Saatman, K.E. (2010). Temporal and regional changes in IGF-1/IGF-1R signaling in the mouse brain after traumatic brain injury. J Neurotrauma *27*, 95-107.

Maeda, A., Crabb, J.W., and Palczewski, K. (2005). Microsomal glutathione S-transferase 1 in the retinal pigment epithelium: protection against oxidative stress and a potential role in aging. Biochemistry *44*, 480-489.

Makkinje, A., Quinn, D.A., Chen, A., Cadilla, C.L., Force, T., Bonventre, J.V., and Kyriakis, J.M. (2000). Gene 33/Mig-6, a transcriptionally inducible adapter protein that binds GTP-Cdc42 and activates SAPK/JNK. A potential marker transcript for chronic pathologic conditions, such as diabetic nephropathy. Possible role in the response to persistent stress. J Biol Chem *275*, 17838-17847.

Malenka, R.C., and Nicoll, R.A. (1999). Long-term potentiation--a decade of progress? Science *285*, 1870-1874.

Manaka, S., and Sano, K. (1978). Thyrotropin-Releasing Hormone tartrate (TRH-T)

shortens concussion effects following head impact in mice. Neurosci Lett *8*, 255-258.

Maroon, J.C., Lovell, M.R., Norwig, J., Podell, K., Powell, J.W., and Hartl, R. (2000). Cerebral concussion in athletes: evaluation and neuropsychological testing. Neurosurgery *47*, 659-669; discussion 669-672.

Matser, E.J., Kessels, A.G., Lezak, M.D., Jordan, B.D., and Troost, J. (1999). Neuropsychological impairment in amateur soccer players. JAMA *282*, 971-973.

Matser, J.T., Kessels, A.G., Jordan, B.D., Lezak, M.D., and Troost, J. (1998). Chronic traumatic brain injury in professional soccer players. Neurology *51*, 791-796.

Matser, J.T., Kessels, A.G., Lezak, M.D., and Troost, J. (2001). A dose-response relation of headers and concussions with cognitive impairment in professional soccer players. J Clin Exp Neuropsychol *23*, 770-774.

McIntosh, T.K. (1993). Novel pharmacologic therapies in the treatment of experimental traumatic brain injury: a review. J Neurotrauma *10*, 215-261.

Mizuno, K., Antunes-Martins, A., Ris, L., Peters, M., Godaux, E., and Giese, K.P. (2007). Calcium/calmodulin kinase kinase beta has a male-specific role in memory formation. Neuroscience *145*, 393-402.

Moon, C., Lee, J., Ahn, M., and Shin, T. (2005). Involvement of Disabled-2 protein in the central nervous system inflammation following experimental cryoinjury of rat brains. Neurosci Lett *378*, 88-91.

Muchowski, P.J., and Wacker, J.L. (2005). Modulation of neurodegeneration by molecular chaperones. Nat Rev Neurosci *6*, 11-22.

Muto, S., Aiba, A., Saito, Y., Nakao, K., Nakamura, K., Tomita, K., Kitamura, T., Kurabayashi, M., Nagai, R., Higashihara, E., *et al.* (2002). Pioglitazone improves the phenotype and molecular defects of a targeted Pkd1 mutant. Hum Mol Genet *11*, 1731-1742.

Nag, S., Venugopalan, R., and Stewart, D.J. (2007). Increased caveolin-1 expression precedes decreased expression of occludin and claudin-5 during blood-brain barrier breakdown. Acta Neuropathol *114*, 459-469.

Nagata, Y., and Todokoro, K. (1999). Requirement of activation of JNK and p38 for environmental stress-induced erythroid differentiation and apoptosis and of inhibition of

ERK for apoptosis. Blood *94*, 853-863.

Nakamura, M., Saatman, K.E., Galvin, J.E., Scherbel, U., Raghupathi, R., Trojanowski, J.Q., and McIntosh, T.K. (1999). Increased vulnerability of NFH-LacZ transgenic mouse to traumatic brain injury-induced behavioral deficits and cortical damage. J Cereb Blood Flow Metab *19*, 762-770.

Narayan, R.K., Michel, M.E., Ansell, B., Baethmann, A., Biegon, A., Bracken, M.B., Bullock, M.R., Choi, S.C., Clifton, G.L., Contant, C.F., *et al.* (2002). Clinical trials in head injury. J Neurotrauma *19*, 503-557.

Nikolaev, A., McLaughlin, T., O'Leary, D.D., and Tessier-Lavigne, M. (2009). APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. Nature *457*, 981-989.

Ning, M., Sarracino, D.A., Kho, A.T., Guo, S., Lee, S.R., Krastins, B., Buonanno, F.S., Vizcaino, J.A., Orchard, S., McMullin, D., *et al.* (2011). Proteomic temporal profile of human brain endothelium after oxidative stress. Stroke *42*, 37-43.

Nishimura, Y., Martin, C.L., Vazquez-Lopez, A., Spence, S.J., Alvarez-Retuerto, A.I., Sigman, M., Steindler, C., Pellegrini, S., Schanen, N.C., Warren, S.T., *et al.* (2007). Genome-wide expression profiling of lymphoblastoid cell lines distinguishes different forms of autism and reveals shared pathways. Hum Mol Genet *16*, 1682-1698.

Ohta, Y., Kousaka, K., Nagata-Ohashi, K., Ohashi, K., Muramoto, A., Shima, Y., Niwa, R., Uemura, T., and Mizuno, K. (2003). Differential activities, subcellular distribution and tissue expression patterns of three members of Slingshot family phosphatases that dephosphorylate cofilin. Genes Cells *8*, 811-824.

Okuda, T., Higashi, Y., Kokame, K., Tanaka, C., Kondoh, H., and Miyata, T. (2004). Ndrg1-deficient mice exhibit a progressive demyelinating disorder of peripheral nerves. Mol Cell Biol *24*, 3949-3956.

Papadia, S., Soriano, F.X., Leveille, F., Martel, M.A., Dakin, K.A., Hansen, H.H., Kaindl, A., Sifringer, M., Fowler, J., Stefovska, V., *et al.* (2008). Synaptic NMDA receptor activity boosts intrinsic antioxidant defenses. Nat Neurosci *11*, 476-487.

Parikh, S., Koch, M., and Narayan, R.K. (2007). Traumatic brain injury. Int Anesthesiol Clin *45*, 119-135.

Park, D.S., Obeidat, A., Giovanni, A., and Greene, L.A. (2000). Cell cycle regulators in neuronal death evoked by excitotoxic stress: implications for neurodegeneration and its

treatment. Neurobiol Aging *21*, 771-781.

Park, E., Bell, J.D., and Baker, A.J. (2008). Traumatic brain injury: can the consequences be stopped? CMAJ *178*, 1163-1170.

Petchprapai, N., and Winkelman, C. (2007). Mild traumatic brain injury: determinants and subsequent quality of life. A review of the literature. J Neurosci Nurs *39*, 260-272.

Peters, M., Mizuno, K., Ris, L., Angelo, M., Godaux, E., and Giese, K.P. (2003). Loss of Ca2+/calmodulin kinase kinase beta affects the formation of some, but not all, types of hippocampus-dependent long-term memory. J Neurosci *23*, 9752-9760.

Piantadosi, C.A., and Zhang, J. (1996). Mitochondrial generation of reactive oxygen species after brain ischemia in the rat. Stroke *27*, 327-331; discussion 332.

Popovich, P.G., and Jones, T.B. (2003). Manipulating neuroinflammatory reactions in the injured spinal cord: back to basics. Trends Pharmacol Sci *24*, 13-17.

Raghupathi, R., Conti, A.C., Graham, D.I., Krajewski, S., Reed, J.C., Grady, M.S., Trojanowski, J.Q., and McIntosh, T.K. (2002). Mild traumatic brain injury induces apoptotic cell death in the cortex that is preceded by decreases in cellular Bcl-2 immunoreactivity. Neuroscience *110*, 605-616.

Raghupathi, R., Fernandez, S.C., Murai, H., Trusko, S.P., Scott, R.W., Nishioka, W.K., and McIntosh, T.K. (1998). BCL-2 overexpression attenuates cortical cell loss after traumatic brain injury in transgenic mice. J Cereb Blood Flow Metab *18*, 1259-1269.

Roberts, G.W., Whitwell, H.L., Acland, P.R., and Bruton, C.J. (1990). Dementia in a punch-drunk wife. Lancet *335*, 918-919.

Saatman, K.E., Duhaime, A.C., Bullock, R., Maas, A.I., Valadka, A., and Manley, G.T. (2008). Classification of traumatic brain injury for targeted therapies. J Neurotrauma *25*, 719-738.

Saatman, K.E., Graham, D.I., and McIntosh, T.K. (1998). The neuronal cytoskeleton is at risk after mild and moderate brain injury. J Neurotrauma *15*, 1047-1058.

Sabel, B.A., Dunbar, G.L., and Stein, D.G. (1984). Gangliosides minimize behavioral deficits and enhance structural repair after brain injury. J Neurosci Res *12*, 429-443.

Saito, N., Okada, Y., Noda, Y., Kinoshita, Y., Kondo, S., and Hirokawa, N. (1997). KIFC2

is a novel neuron-specific C-terminal type kinesin superfamily motor for dendritic transport of multivesicular body-like organelles. Neuron *18*, 425-438.

Sandberg Nordqvist, A.C., von Holst, H., Holmin, S., Sara, V.R., Bellander, B.M., and Schalling, M. (1996). Increase of insulin-like growth factor (IGF)-1, IGF binding protein-2 and -4 mRNAs following cerebral contusion. Brain Res Mol Brain Res *38*, 285-293.

Saville, M.K., Sparks, A., Xirodimas, D.P., Wardrop, J., Stevenson, L.F., Bourdon, J.C., Woods, Y.L., and Lane, D.P. (2004). Regulation of p53 by the ubiquitin-conjugating enzymes UbcH5B/C in vivo. J Biol Chem *279*, 42169-42181.

Schmit, F., Cremer, S., and Gaubatz, S. (2009). LIN54 is an essential core subunit of the DREAM/LINC complex that binds to the cdc2 promoter in a sequence-specific manner. FEBS J *276*, 5703-5716.

Sepulveda, J.L., and Wu, C. (2006). The parvins. Cell Mol Life Sci *63*, 25-35.

Shannon, P., Smith, C.R., Deck, J., Ang, L.C., Ho, M., and Becker, L. (1998). Axonal injury and the neuropathology of shaken baby syndrome. Acta Neuropathol *95*, 625-631.

Shapira, Y., Shohami, E., Sidi, A., Soffer, D., Freeman, S., and Cotev, S. (1988). Experimental closed head injury in rats: mechanical, pathophysiologic, and neurologic properties. Crit Care Med *16*, 258-265.

Shear, D.A., Tate, M.C., Archer, D.R., Hoffman, S.W., Hulce, V.D., Laplaca, M.C., and Stein, D.G. (2004). Neural progenitor cell transplants promote long-term functional recovery after traumatic brain injury. Brain Res *1026*, 11-22.

Shibahara, K., Asano, M., Ishida, Y., Aoki, T., Koike, T., and Honjo, T. (1995). Isolation of a novel mouse gene MA-3 that is induced upon programmed cell death. Gene *166*, 297-301.

Shimura, H., Schwartz, D., Gygi, S.P., and Kosik, K.S. (2004). CHIP-Hsc70 complex ubiquitinates phosphorylated tau and enhances cell survival. J Biol Chem *279*, 4869-4876.

Silverstone, P.H., Williams, R., McMahon, L., Fleming, R., and Fogarty, S. (2008). Convulsive liability of bupropion hydrochloride metabolites in Swiss albino mice. Ann Gen Psychiatry *7*, 19.

Siuciak, J.A., McCarthy, S.A., Chapin, D.S., and Martin, A.N. (2008). Behavioral and neurochemical characterization of mice deficient in the phosphodiesterase-4B (PDE4B) enzyme. Psychopharmacology (Berl) *197*, 115-126.

Slemmer, J.E., Matser, E.J., De Zeeuw, C.I., and Weber, J.T. (2002). Repeated mild injury causes cumulative damage to hippocampal cells. Brain *125*, 2699-2709.

Slemmer, J.E., and Weber, J.T. (2005). The extent of damage following repeated injury to cultured hippocampal cells is dependent on the severity of insult and inter-injury interval. Neurobiol Dis *18*, 421-431.

Smith, D.H., Chen, X.H., Pierce, J.E., Wolf, J.A., Trojanowski, J.Q., Graham, D.I., and McIntosh, T.K. (1997). Progressive atrophy and neuron death for one year following brain trauma in the rat. J Neurotrauma *14*, 715-727.

Smith, D.H., Soares, H.D., Pierce, J.S., Perlman, K.G., Saatman, K.E., Meaney, D.F., Dixon, C.E., and McIntosh, T.K. (1995). A model of parasagittal controlled cortical impact in the mouse: cognitive and histopathologic effects. J Neurotrauma *12*, 169-178.

Soriano, F.X., Leveille, F., Papadia, S., Higgins, L.G., Varley, J., Baxter, P., Hayes, J.D., and Hardingham, G.E. (2008). Induction of sulfiredoxin expression and reduction of peroxiredoxin hyperoxidation by the neuroprotective Nrf2 activator 3H-1,2-dithiole-3-thione. J Neurochem *107*, 533-543.

Sowa, M.E., Bennett, E.J., Gygi, S.P., and Harper, J.W. (2009). Defining the human deubiquitinating enzyme interaction landscape. Cell *138*, 389-403.

Sreedharan, S., Stephansson, O., Schioth, H.B., and Fredriksson, R. (2010). Long evolutionary conservation and considerable tissue specificity of several atypical solute carrier transporters. Gene.

Stoica, B.A., Movsesyan, V.A., Knoblach, S.M., and Faden, A.I. (2005). Ceramide induces neuronal apoptosis through mitogen-activated protein kinases and causes release of multiple mitochondrial proteins. Mol Cell Neurosci *29*, 355-371.

Sultana, R., Banks, W.A., and Butterfield, D.A. (2010). Decreased levels of PSD95 and two associated proteins and increased levels of BCl2 and caspase 3 in hippocampus from subjects with amnestic mild cognitive impairment: Insights into their potential roles for loss of synapses and memory, accumulation of Abeta, and neurodegeneration in a prodromal stage of Alzheimer's disease. J Neurosci Res *88*, 469-477.

Sun, W., Park, K.W., Choe, J., Rhyu, I.J., Kim, I.H., Park, S.K., Choi, B., Choi, S.H., Park, S.H., and Kim, H. (2005). Identification of novel electroconvulsive shock-induced and activity-dependent genes in the rat brain. Biochem Biophys Res Commun *327*, 848-856.

Tanaka, S., Kitagawa, K., Ohtsuki, T., Yagita, Y., Takasawa, K., Hori, M., and Matsumoto, M. (2002). Synergistic induction of HSP40 and HSC70 in the mouse hippocampal neurons after cerebral ischemia and ischemic tolerance in gerbil hippocampus. J Neurosci Res *67*, 37-47.

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

Tasaki, T., Mulder, L.C., Iwamatsu, A., Lee, M.J., Davydov, I.V., Varshavsky, A., Muesing, M., and Kwon, Y.T. (2005). A family of mammalian E3 ubiquitin ligases that contain the UBR box motif and recognize N-degrons. Mol Cell Biol *25*, 7120-7136.

Teasdale, G., and Jennett, B. (1974). Assessment of coma and impaired consciousness. A practical scale. Lancet *2*, 81-84.

Tegner, Y., and Lorentzon, R. (1996). Concussion among Swedish elite ice hockey players. Br J Sports Med *30*, 251-255.

TheManagementofConcussion/mTBIWorkingGroup (2009). VA/DoD CLINICAL PRACTICE GUIDELINE FOR MANAGEMENT OF CONCUSSION/ MILD TRAUMATIC BRAIN INJURY, D.o.V. Affairs, and D.o. Defense, eds.

Thurman, D.J., Alverson, C., Dunn, K.A., Guerrero, J., and Sniezek, J.E. (1999). Traumatic brain injury in the United States: A public health perspective. J Head Trauma Rehabil *14*, 602-615.

Tim Anderson, Marcus Heitger, and Macleod, A.D. (2006). Concussion and mild head injury. Pract Neurol, 342-357.

Tom Tang, Y., Emtage, P., Funk, W.D., Hu, T., Arterburn, M., Park, E.E., and Rupp, F. (2004). TAFA: a novel secreted family with conserved cysteine residues and restricted expression in the brain. Genomics *83*, 727-734.

Tran, H., Hamada, F., Schwarz-Romond, T., and Bienz, M. (2008). Trabid, a new positive regulator of Wnt-induced transcription with preference for binding and cleaving

K63-linked ubiquitin chains. Genes Dev *22*, 528-542.

Trifonov, S., Houtani, T., Shimizu, J., Hamada, S., Kase, M., Maruyama, M., and Sugimoto, T. GPR155: Gene organization, multiple mRNA splice variants and expression in mouse central nervous system. Biochem Biophys Res Commun *398*, 19-25.

Valadka, A.B. (2004). Injury to the cranium (New York, McGraw-Hill,Medical Pub.Division).

VanGuilder, H.D., Yan, H., Farley, J.A., Sonntag, W.E., and Freeman, W.M. (2010). Aging alters the expression of neurotransmission-regulating proteins in the hippocampal synaptoproteome. J Neurochem *113*, 1577-1588.

Vodermaier, H.C. (2004). APC/C and SCF: controlling each other and the cell cycle. Curr Biol *14*, R787-796.

Wang, Y., Branicky, R., Stepanyan, Z., Carroll, M., Guimond, M.P., Hihi, A., Hayes, S., McBride, K., and Hekimi, S. (2009). The anti-neurodegeneration drug clioquinol inhibits the aging-associated protein CLK-1. J Biol Chem *284*, 314-323.

Weber, J.T. (2004). Calcium homeostasis following traumatic neuronal injury. Curr Neurovasc Res *1*, 151-171.

Wildburger, R., Zarkovic, N., Leb, G., Borovic, S., Zarkovic, K., and Tatzber, F. (2001). Post-traumatic changes in insulin-like growth factor type 1 and growth hormone in patients with bone fractures and traumatic brain injury. Wien Klin Wochenschr *113*, 119-126.

Wu, Y.P., Mizugishi, K., Bektas, M., Sandhoff, R., and Proia, R.L. (2008). Sphingosine kinase 1/S1P receptor signaling axis controls glial proliferation in mice with Sandhoff disease. Hum Mol Genet *17*, 2257-2264.

Xie, J., Lee, J.A., Kress, T.L., Mowry, K.L., and Black, D.L. (2003). Protein kinase A phosphorylation modulates transport of the polypyrimidine tract-binding protein. Proc Natl Acad Sci U S A *100*, 8776-8781.

Yokota, T. (2009). [MicroRNA and central nervous system]. Brain Nerve *61*, 167-176.

Yoon, S., Cong, W.T., Bang, Y., Lee, S.N., Yoon, C.S., Kwack, S.J., Kang, T.S., Lee, K.Y., Choi, J.K., and Choi, H.J. (2009). Proteome response to ochratoxin A-induced apoptotic cell death in mouse hippocampal HT22 cells. Neurotoxicology *30*, 666-676.

Zhang, D., and Richardson, D.R. (2011). Endoplasmic reticulum protein 29 (ERp29): An emerging role in cancer. Int J Biochem Cell Biol *43*, 33-36.

Zhang, G.R., Cheng, X.R., Zhou, W.X., and Zhang, Y.X. (2008a). Age-related expression of STUB1 in senescence-accelerated mice and its response to anti-Alzheimer's disease traditional Chinese medicine. Neurosci Lett *438*, 371-375.

Zhang, H.T., Huang, Y., Masood, A., Stolinski, L.R., Li, Y., Zhang, L., Dlaboga, D., Jin, S.L., Conti, M., and O'Donnell, J.M. (2008b). Anxiogenic-like behavioral phenotype of mice deficient in phosphodiesterase 4B (PDE4B). Neuropsychopharmacology *33*, 1611-1623.

Zhang, Y., Luan, Z., Liu, A., and Hu, G. (2001). The scaffolding protein CASK mediates the interaction between rabphilin3a and beta-neurexins. FEBS Lett *497*, 99-102.

Zheng, H., Lapointe, J., and Hekimi, S. (2010). Lifelong protection from global cerebral ischemia and reperfusion in long-lived Mclk1(+/)(-) mutants. Exp Neurol *223*, 557-565.

Zheng, Y., Cheng, X.R., Zhou, W.X., and Zhang, Y.X. (2008). Gene expression patterns of hippocampus and cerebral cortex of senescence-accelerated mouse treated with Huang-Lian-Jie-Du decoction. Neurosci Lett *439*, 119-124.

Zhou, J., Liyanage, U., Medina, M., Ho, C., Simmons, A.D., Lovett, M., and Kosik, K.S. (1997). Presenilin 1 interaction in the brain with a novel member of the Armadillo family. Neuroreport *8*, 2085-2090.

Zhu, Y., Kakinuma, N., Wang, Y., and Kiyama, R. (2008). Kank proteins: a new family of ankyrin-repeat domain-containing proteins. Biochim Biophys Acta *1780*, 128-133.