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Behavioral consequences of minimal traumatic brain injury in mice

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Victims of minor traumatic brain injury (mTBI), who show no clear morphological brain defects, frequently manifest cognitive, behavioral and emotional difficulties that can be long-lasting. In this paper we present a modified weight drop model used to deliver a closed head minimal traumatic brain injury to mice, which closely mimics real-life injuries and the symptoms observed in mTBI patients. Our choice of impact force does not produce structural damage to the brain and its surrounding tissue (as examined by MRI), any skull fracture, no edema and no evident damage to the blood-brain barrier (BBB). Moreover, our mTBI mice show no abnormal behavior on recovering from the weight drop, or any change in other brain functions such as reflexes, balance, exploration, strength, locomotor activity and swim speed. Since our mTBI model does not produce neurological, motor or sensory damage to the mice, it allows the direct evaluation of mTBI sequelae on the mice behavior and cognitive abilities. Using a variety of cognitive and behavioral tests (Morris water maze, staircase test, passive avoidance test, water T-maze, hot palate, elevated plus maze and forced swimming test) we assessed the short- and long-term sequelae induced by our model. Our results indicate that our closed head mTBI cause profound and long-lasting, irreversible learning and memory impairments, accompanied by a depressive-like behavior in mice that are evident even 90 days post injury. Our results indicate that the closed head mTBI model presented here may be useful in the development of novel therapeutic approaches, such as neuroprotective agents, for mTBI.

Key words: forced swimming test, Morris water maze, mTBI, passive avoidance, swim T-maze

INTRODUCTION

Traumatic brain injury (TBI) is a frequent injury in victims of sports and in motor vehicle accident especially for young men. One third of the 2 million TBI victims admitted to hospitals each year in the United States are children and young adults (Berger et al. 1999). TBI is a diagnosis encompassing a broad range of short and long-term physical, cognitive, behavioral and emotional impairments that depend on the severity of the injury (Waxweiler et al. 1995, Berger et al. 1999, Albensi 2001). Severe TBI victims are relatively easy to diagnose since they suffer clear pathologies such as direct brain tissue damage, rapture of the blood-brain barrier and post injury edema. On the other hand, patients sustaining moderate to minimal TBI (mTBI) are more difficult to diagnose (Kibby and Long 1996). Routine and extended clinical and laboratory evaluations of mTBI patients often fails to show clear morphological brain defects, however these patients frequently suffer from lasting cognitive, behavioral and emotional difficulties including various degrees of amnesia, altered executive functions, difficulty in concentration, depression, apathy, and anxiety (Levin et al. 1987, Kibby and Long 1996, Arciniegas et al. 1999). Clinically, mTBI is often resolved within the first year after injury, but many of the patients continue to manifest prolonged (and some even permanent) neurocognitive dysfunctions, a condition termed "post-concussive syndrome" (Hamm et al. 1993, Finset et al. 1999, Margulies 2000).

The pathophysiology of TBI is complex and can be roughly divided into primary and secondary damage. The primary damage is severity dependent and may

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include direct damage to the brain tissue, diffuse and direct axonal damage, rupture of the blood-brain barrier, hemorrhage, edema and skull fractures (Shapira et al. 1993, Assaf et al. 1999, Graham et al. 2000). Studies utilizing various experimental models of TBI indicate that the primary brain damage triggers cellular pathologies, which in turn lead to long-lasting secondary neuronal and glial damage (Pierce et al. 1996, 1998, Berger et al. 1999, Graham et al. 2000, Albensi 2001). The secondary damage to the brain comprises complex interconnecting structural, functional, cellular and molecular changes. One of the earliest secondary damage of TBI is abnormal extra- and intra-cellular chemical homeostasis. Following injury, large quantities of excitatory neurotransmitters including glutamate and aspartate are released into the extracellular space of the affected area in a severity-dependent manner (Faden et al. 1989, Albensi 2001, Ustun et al. 2001). The large glutamate release (which may last up to 30 minutes) causes a repeated and non-discriminatory activation of ionic and metabotrophic glutamate channels in both neurons and glia. This large uncontrolled transmitter release disturbs the brain's delicate ionic balance between the intracellular calcium, sodium and magnesium and the extracellular potassium (Faden et al. 1989, Laurer and McIntosh 1999, Graham et al. 2000). Beyond a certain degree, the intracellular increase of sodium and calcium activates a vicious cycle in the neurons that further depolarize the cells and potentiate the increase in glutamate release, leading to further excito-toxicity. Uncontrolled changes in calcium concentration can have special devastating effects on neurons since many of their functions are tightly regulated by calcium concentration. For example, elevated levels of intracellular calcium interfere with mitochondria oxidative phosphorylation, which in turn leads to oxidative stress that activates apoptosis. TBI also interferes with the normal regulation of various genes such as immediate early genes and apoptosis regulating genes (Abrous et al. 1999, Napieralski et al. 1999, Yakovlev et al. 2001).

Several experimental models were developed for the study of traumatic brain injury including fluid percussion, rigid indentation, and weight drop models (Laurer and McIntosh 1999). The most commonly used model is the fluid percussion in which the exposed dura matter is injured by a precise impact of a short fluid pressure pulse (Dixon et al. 1988, Cortez et al. 1989). In the rigid indentation model a precise mechanical injury is delivered to the exposed dura of a restrained animal head (Lighthall 1988). Weight drop models are mainly used to deliver severe TBI *via* a free falling weight dropped onto the animal's skin-free skull, which usually produces a high number of skull fractures (Shapira et al. 1993, Engelborghs et al. 1998).

Most real-life TBI brain injuries are moderate to minimal in severity, and are the result of a strike to the intact head. However, current models of traumatic brain injury are mostly used to deliver severe TBI, which produces complex pathologies, rendering the study of refined cellular responses to the injury difficult. Here we present a modified weight drop model used to deliver a closed head minimal TBI to mice, which closely mimics symptoms observed in human "post-concussive syndrome" (Pan et al. 2003, Zohar et al. 2003, Milman et al. 2005). We used a variety of cognitive and behavioral tests to assess the sequelae induced by our model including; Morris water maze, water T-maze, staircase test, passive avoidance test, and forced swimming test. Our results indicate that our minimal closed head TBI causes profound and long-lasting learning and memory impairments in mice. The observed cognitive deficits were not accompanied by neurological damage, damage to the blood-brain barrier, brain edema or gross anatomical changes to the brain (as measured by MRI).

METHODS

Mice

Male ICR mice weighing 30-40 g were kept 2-5 *per* cage under a constant 12 hours light/dark cycle, at room temperature. Food (Purina rodent chow) and water were available *ad libitum*. For each time point, in each experimental group (7, 30, 60 and 90 days after injury), at least 10 different randomized mice served as experimental subjects and at least 10 mice as control. Each mouse was used for one experiment only and at one time point, because the mice should not be familiar with either the cognitive or behavioral tests they perform. The ethics committee of the Sackler Faculty of Medicine approved the experimental protocols M-98-014 and M-08-040, in compliance with the guidelines for animal experimentation of the National Institutes of Health (DHEW publication 85-23, revised, 1995). Throughout the studies the minimal possible number of animals was used and all efforts were made to minimize their suffering.

Induction of closed-head concussive mTBI

Experimental mild traumatic brain injury was induced using the concussive head trauma device described previously (Pan et al. 2003, Zohar et al. 2003). Mice, slightly anesthetized by isoflurane were situated under a device consisting of a metal tube (inner diameter 13 mm) placed vertically over the animal's head. The injury was induced by dropping a 20, 25 or 30 gr metal weight from 80 cm height down the metal tube, striking the skull. The animals were held in such a way that the impact on the skull was anteriolaterally just anterior to the right ear. A sponge immobilization board was supporting the head to allow some head movements during the injury, analogously to the movements occurring during closed head injury in car accidents. Immediately after the injury, mice were returned to their cages for recovery. After the injury, the animals did not exhibit any apnea. The effect of the injury was studied at 7, 30, 60 and 90 days following the trauma, using different groups of mice for each time point and for each test (at least 10 mice in a group). Sham control mice were slightly anesthetized and put in the head trauma device without receiving any injury.

Well being of mice suffered mTBI

The neurological status of the experimental mice was assessed using an extensive set of tests, 1 and 24 hours following the injury. The tests included hind-leg flexion reflex (when raised by the tail), righting reflex (of falling on all four legs after a short drop), corneal reflex (blinking response), secretory signs (around the mouth and the eye), strength, beam balance task, beam walking coordination task and exploration and locomotor activity tests, as described previously (Pan et al. 2003, Zohar et al. 2003). In addition, the basic well being of the mice was assessed by testing their motor skills using the staircase test, their pain threshold using the hot plate test and their anxiety level using the elevated plus maze test (see below).

The staircase test

The staircase apparatus (Pan et al. 2003) was constructed from five consecutive identical steps (2.5×10×12.5 cm) with 12.5 cm walls, all made of black Plexiglas. Each test began by placing a mouse on the bottom step, back to the stairs, and then it was allowed to climb the steps for 3 min. Two parameters were measured 1h, 24h, 7, 30 and 60 days post injury: the total number of stairs ascended (NSA) and the number of rearing events (NR). Before each session, the staircase was cleaned with 70% ethanol solution (v/v).

The number of steps climbed and the number of rears were recorded for a 3-minute period. Climbing was defined as a step on which the mouse placed all four paws; rearing was defined as each instance the mouse rose on hind legs anywhere in the apparatus. The number of steps descended was not taken into account. Different groups of mice were used at each time point. Data was analyzed using two-way ANOVA.

Hot plate test

Changes in nociceptive threshold of the injured mice were assessed utilizing the hot plate test 7, 30 and 60 days post injury. The apparatus consists of a metal platform (30×30 cm), capable of being uniformly heated by an electrical current, and is surrounded by a transparent Plexiglas wall (28 cm) (Pick et al. 1991, Lehner et al. 2010). Each mouse was individually placed on the hot-plate after it was heated to 52ºC (± 1ºC). The latency to the first jump response was measured; mice were not allowed to stay on the heated plate for more than 40 seconds, to avoid any damage to their paws. Data was analyzed using two-way ANOVA.

Elevated plus maze

Anxiety level was assessed utilizing the elevated plus maze 7, 30 and 60 days post injury (Alcalay et al. 2004). The apparatus consisted of two open arms (30×5×15 cm) and two closed arms (30×5×15 cm) with an open roof, arranged such that the two arms of each type was opposite each other (in a "+" shape). The maze was elevated 60 cm above the floor level (Hogg 1996). On the test day, mice were placed in the center of the plus-maze, facing one of the open arms. The time spent in the open arm was measured for five minutes. The maze was cleaned between animals with 70% ethanol solution (v/v).

Morris water maze

MWM was used to assess the spatial cognitive abilities of mice after injury. The maze comprised of a circular plastic pool of reduced size to accommodate

for the learning deficits of the injured mice (90 cm in diameter and 60 cm deep), with a featureless white inner surface. The pool was filled with 40 cm of tepid water, made non-transparent with milk powder. The perimeter of the pool was marked with four equally spaced starting positions, dividing it into four imaginary quadrants. A hidden clear Plexiglas platform (10×10 cm) was submerged just under the water level in the center of one of the quadrants.

Testing began by placing the mice at a random starting position, facing the pool wall, allowing them to swim and self discover the location of the escape platform and climb onto it. Whenever a mouse failed to find the platform within 80 sec, it was gently guided to it. Once the mouse located and climbed on the platform, it was permitted to remain there for 20 sec. Each animal's path was tracked by a computerizing video system (HVS Image - Tracker vp-200, Buckingham, UK) and its escape latency was recorded. Mice were trained at 6 trials per day, for 4 consecutive days. On day five, the platform was removed and the mice's memory retention abilities was probed by allowing them to free swim for 80 sec, and the number of times mice crossed the missing platform position and time spent swimming in the missing platform quadrant was recorded. The swimming speed of the mice was calculated by dividing the total swim length by the swim time at the free swim trial. Mouse performance in the acquisition and the memory phases of the MWM were analyzed using repeated measure one-way ANOVA (SigmaStat 3.5).

Passive avoidance task

The passive avoidance task was used to assess simple non-spatial learning ability. The passive avoidance device (Hogg et al. 1998a,b) comprised of two adjoining compartments, one illuminated and one darkened, divided by a guillotine door. The dimensions of each compartment were 30.5×20.5×19 cm. The floor of the compartments consisted of steel rods capable of delivering a slight electric foot shock (0.7 mA for 2 sec) to the animal (Gemini Avoidance System, San Diego Instruments, San Diego, USA).

The passive avoidance task was conducted on days 6 and 7, or 29 and 30, or 59 and 60, or 89 and 90 post injury. The test began by placing the mouse in the lighted compartment, and latency for crossing to the dark compartment was recorded. As soon as the animal crossed into the dark compartment the guillotine door was closed, and the animal received a slight electric foot shock. Following the electric shock, animals were removed from the apparatus and returned to their home cage. Twenty four hours after the electric shock, mice were tested for the retention of the passive avoidance response. Upon testing, mice were placed in the illuminated compartment and the latency for the animal to cross into the dark previously shocked compartment, was recorded. During testing, no shock was delivered to the mice. Normal memory response of 3 minutes latency of no crossing to the dark compartment was assigned as normal response. Data was analyzed using two-way ANOVA.

Swim T-maze

The swim T-maze task was used to assess working memory and learning contribution of the hippocampus (Heinrichs et al. 1996). The swim T-maze device consisted of a three-arm, walled T-maze (60 cm along the stem, 80 cm sides at the T-intersection, 40 cm high walls, with 10-cm-wide passages) made of white Plexiglas. The maze was situated in one corner of a brightly lit testing room. Before testing, the maze was filled with water (21ºC) and 8 cm from the end of one arm (goal arm) a 3-cm-square platform rising from the floor of the maze was submerged just below the water line (Heinrichs et al. 1996).

Mice were tested in the swim T-maze 7, 30, 60 and 90 days post injury. One day prior to training, mice were placed in the maze and allowed to swim for 60 sec with no platform present. The platform was then inserted in the predetermined position at the end of the goal arm, and each mouse was placed directly on the platform for 30 sec. Finally, each mouse was placed at the beginning of the stem and allowed to locate the submerged goal arm platform. The latency to reach the platform and the number of correct choices, defined as entry into the goal arm with a platform were measured during these free choice trials. Each animal was allowed to remain on the platform for 15 sec and then returned to the home cage. Mice, which failed to locate the platform within 1 min, were assigned a latency of 60 sec and placed atop the platform. To determine the effect of injury on memory and learning in the swim T-maze task, repeated measures ANOVA was performed.

Forced swimming test (Porsolt test)

This task was used to assess a depressive-like state in ther injured mice. The forced swimming test apparatus consisted of a clear Plexiglas cylinder (height 25 cm, diameter 10 cm) containing 6 cm of water at 21ºC (Porsolt et al. 1977, Badowska-Szalewska et al. 2009). Mice were put into the cylinders 7, 30, 60 and 90 days after the injury, and left there for 6 min. Because little immobility is observed during the first 2 min, only the movements occurring during the last 4 min was counted. A mouse was judged to be immobile when it ceased trying to swim and remained floating motionless in the water making only those movements necessary to keep its head above water. Data was analyzed using two-way ANOVA.

RESULTS

Well being of mTBI injured mice

The mTBI did not affect the basic well being of all the experimental groups up to 90 days post injury. All neurological function tested for the injured mice were normal (hind-leg flexion reflex, righting reflex, corneal reflex, secretory signs, strength, beam balance task, beam walking coordination task and exploration and locomotor activity tests). Moreover, the performance of the injured mice in the staircase test (indicative of motor skill), the hot plate test (indicative of pain threshold), and the elevated plus maze (indicative of anxiety state) were all normal.

Morris Water Maze

All experimental animals acquired the MWM learning task as demonstrated by their ability to significantly reduce their escape latencies following training ($p<0.01$; Fig 1). However, mice that suffered mTBI showed profound learning and memory deficits. The injured mice of all groups could hardly improve their performance beyond the second trial day, while control mice improved their performance over all the 5-day trial period ($p<0.001$, between each injured group and control from trial day 2 and thereafter). Mice who suffered 30 g injury could not improve their performance linearly and reached a learning plateau after the third experimental learning day, with the exception of the 90 days group.

At the end of their learning trials, control mice improved their learning by 77% relative to their initial escape latency. Interestingly, we found that the learning of the injured mice had a limit of about 40% of their initial escape latency, beyond which they could not improve their performance.

In the MWM free swim trial all the injured mice showed significant impairments in the task performance. Although the swim speed of the injured mice did not change, they crossed the missing platform location significantly less than control mice during the test period ($p<0.05$). These results are consistent with the effects of mTBI on the mouse escape latencies. In the probe trial, all the injured mice showed significant memory retention impairment. Although the swim speed of the injured mice did not change, they crossed the missing platform location significantly less than control mice at all the test periods (Control 4.2 ± 0.06 sec, 7d - 3 ± 0.1 sec, 30d - 3.2 ± 0.11 sec, 60d - 3.5 ± 0.06 sec, 90d - 2.8 ± 0.04 sec; $p<0.05$). These results are consistent with the effects of mTBI on the mouse escape latencies.

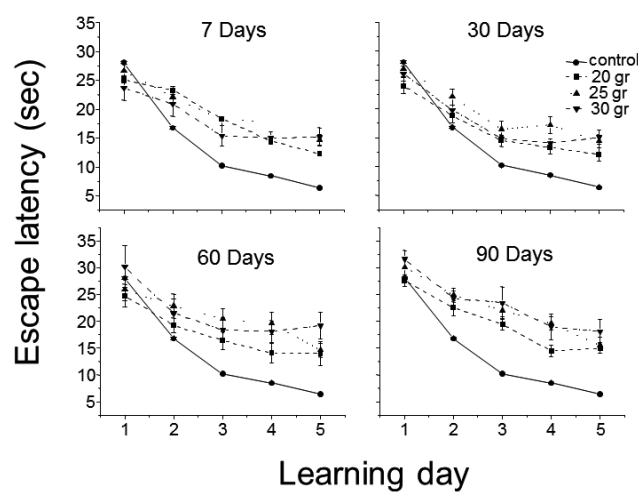

Fig. 1. Time- and force-dependent spatial learning deficits in brain-injured mice following 20, 25, and 30 g weight drop. Please note the force-dependent long-term effect of the injury and the inability to improve performance beyond 50% of the initial escape latency. Since all control animals in the experimental groups showed no significant difference, they were pooled and statistics were calculated between the specific experimental groups and the pooled control results.

Passive avoidance task

During performance on the passive avoidance task, an interaction between group and experimental day was found to be almost a significant factor in determining the passive avoidance learning (p=0.074 by two-way ANOVA, group (injured or control) × time). A significant difference between the groups was found (p<0.001; two-way ANOVA, group × time). The ability of control mice to learn the passive avoidance as measured by the test latency was similar in all the test days. On the other hand, the injured mice ability to learn the task was significantly decreased 30 days post injury (significance after Bonferroni's multiple comparison tests, p<0.05) when compared to controls. The same tendency of learning was observed on days 60 and 90 although it did not reach significance (Fig. 2).

Swim T-maze

The performance in the swim T-maze on days 7, 30, 60 and 90 post-injury was evaluated by the percentage of correct arm choice and analyzed by a repeated measure ANOVA for each test day. Significant learning deficits were observed in all time-points (days 7, 30, 90; p<0.001 and day 60; p<0.005). However, a significant time × group = interaction was found only at 30 days post-injury (p<0.001). The main statistical effect of group is significant only at the 30 test day (p<0.001). Injured mice showed fewer correct choices than controls on test days longer than 30 days post-injury; reaching significance on test days 4 and 5 (Bonferroni's multiple comparison test, p<0.05). The latency to climb onto the T-maze platform was also affected by experimental day (p<0.001), however there was no group main effect for a specific time point (p>0.05). This suggests that there was no significant difference in swim speed between the two groups. No interaction between group and experimental day was found, meaning groups' performance was not different over the test days (Fig. 3).

Forced swimming test

In the forced swim test, swimming activity of mice is measured starting 2 min after they are placed in the water filled forced swim cylinder. The measure is the percentage of time they spent in immobility.

Using two-way ANOVA (group × time), we found a significant difference between the injured and the control groups (p<0.001). Furthermore, we found a statistical influence of the time points (p=0.025). No interaction was found between group and experimental day, meaning activity did not change over time (p>0.05).

In all time points, the injured groups spent more time in the immobile state than the sham-control group. *Post-hoc* analysis using Bonferroni's multiple comparison tests found a significant difference between the two groups on test day 7 (p=0.04) and on test day 90 (p=0.004; Fig. 4).

DISCUSSION

TBI is a growing public health concern that affects mostly young men. Over 90% of TBI patients are defined as suffering from mild TBI, with symptoms that include short- and long- term cognitive and emotional sequelae.

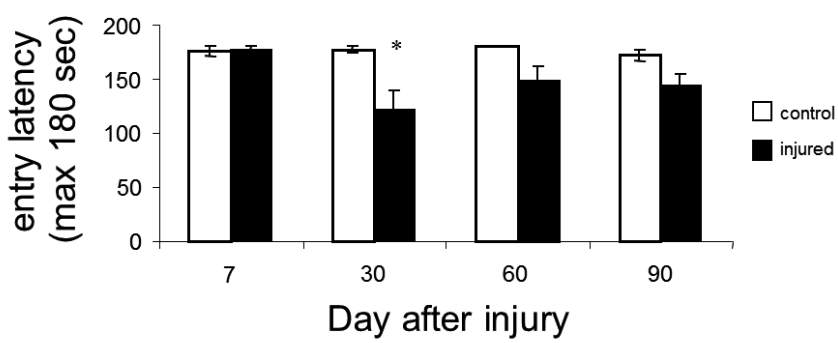

Fig. 2. Mean time (sec) that the mice spent out of the electrifying area, as measured in the passive avoidance paradigm, at different times after the injury (maximum time 180 sec). A two-way ANOVA on the differences between the test latency (day 7, 30, 60, and 90) indicated that injured and sham-injured animals almost differentially change over test days (p=0.074). There is a significant difference between the groups (p<0.001; i.e., only the sham-injured group avoided entering the chamber in which they had been shocked). Injured mice ability to learn the task was significantly reduced on test day 30 from control (*p<0.05 by Bonferroni's multiple comparison tests). Values are mean ± SEM (n>10 in each group)

Focal impact to the head may lead to traumatic brain injury the consequences of which vary with the damage inflicted. The initial mechanical impact can vary from superficial bruises to the head, to skull fracturing and direct damage to the brain. The primary damage to the brain often leads to secondary cascade of events such as breakdown of the dura-mater and the blood-brain barrier, edema, inflammation, change in ionic homeostasis, excitotoxicity, apoptosis and pathological activation of various genes and biochemical processes (Lyeth et al. 1993, Laurer and McIntosh 1999, Tashlykov et al. 2007, 2009, Tweedie et al. 2007). The nature and extend of the secondary damage to the brain are still poorly understood, and may have long term consequences to TBI patients suffering from all levels of injury.

The multiple factors involved in brain injury induce many complications that may mask each other. In order to study the role of the injury to the brain by itself with no peripheral damage, we developed a closed-head non-invasive weight drop brain injury model, which does not include any surgical intrusions nor deep anesthesia. In several studies we have demonstrated that our model produce brain injury sequelea similar to those observed in mild traumatic brain injured patients. The mTBI induced in the mice, did not produce external damage to the brain and its surrounding tissue (as examined by MRI), any skull fracture, no edema and no evident damage to the BBB (Pan et al. 2003, Zohar et al. 2003). However, although no peripheral damage was induced to the brain we demonstrated that the injury induce apoptosis to neurons in brain areas such as the hippocampus and the cortex (Strugar et al. 1993, Tweedie et al. 2007, Tashlykov et al. 2009). Moreover, our minimally brain injured mice showed no abnormal behavior on recovering from the weight drop, or any damage to other brain functions such as reflexes, balance, exploration, strength, locomotor activity and swim speed. Moreover, the mTBI did not affect the mice's motor activity, pain threshold, nor anxiety (tested by the staircase test, the hot plate test and the elevated plus maze task accordingly), indicating the injury did not affect their well being.

Since our mTBI model did not produce neurological, motor or sensory damage to the mice, it allowed us to directly evaluate the sequalea of mTBI on the mice behavior and cognitive abilities.

Our MWM results indicate that mTBI can produce significant and irreversible long-term deficits to the mice behavior and cognitive abilities. Farther, we found the injured mice to suffer from spatial and non-spatial learning and memory impairments and from depressive-like behaviors. The mTBI mice had limited ability to improve their performance in learning the MWM and needed significantly more time than con-

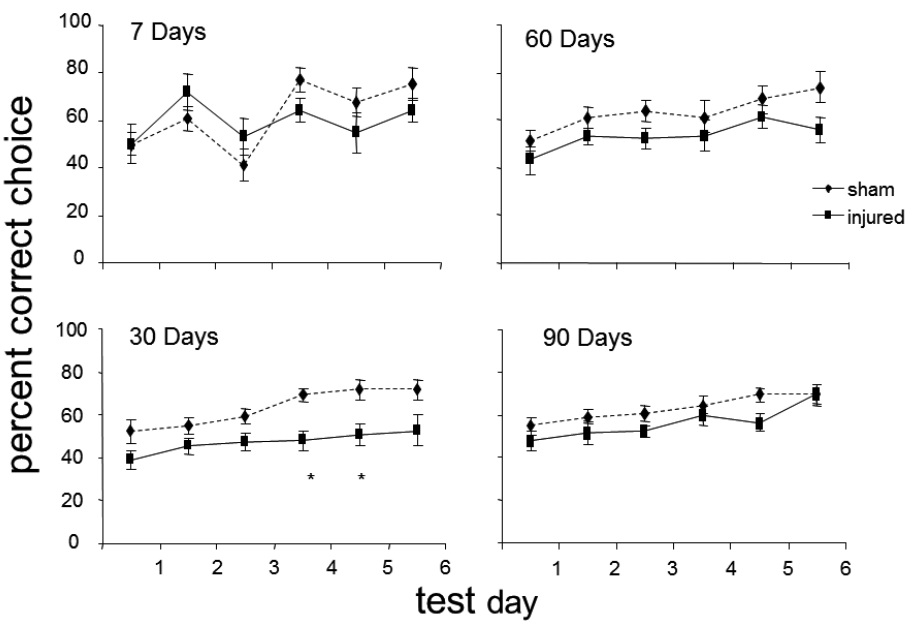

Fig. 3. Percent of correct choice as a function of the test day, as measured in the swim T-maze apparatus, at 7, 30, 60, and 90 days post-injury. Each day consists of eight trails for each mouse (n>10 in each group). Values are mean ± SEM. Significance after Bonferroni's multiple comparison tests: *p<0.05.

trol to find the submerged platform. Moreover, the injured mice could not improve their escape latency by more than 40% of their initial performance, a criterion that was usually met by control mice after the third trial day. Similarly, our mTBI had impaired the injured mice cognitive abilities tested by the swim T-maze and by the non-spatial passive avoidance learning task.

An array of evidences suggests that deficits in learning and memory are common sequelae of mild to moderate brain injury in humans (Rimel et al. 1982, Strugar et al. 1993). Here we present evidence that mTBI in mice is associated with impairments in memory and learning as assessed by MWM, passive avoidance and swim T-maze tasks. Morris water maze is a task that relies heavily on spatial memory and its integration with the learning of the task. Therefore, our results suggest that the integration of the learning task is the main process that is impaired by the minimal brain injury. Substantial evidence suggests that rat and mouse performance in the Morris water maze is sensitive to hippocampal and cortex damage, which are also the most vulnerable parts of the brain to various types and degrees of insults (Dixon et al. 1988, Cortez et al. 1989, Willner 1990, Lyeth et al. 1993, Smith et al. 1998). Indeed, our studies indicate that there is a selective, but significant neuronal apoptosis damage that occur in brain areas previously implicated in the acquisition and retention processes involved in this tasks; i.e., the cortex and the hippocampus (Willner 1990, Laurer and McIntosh 1999, Tashlykov et al. 2007, 2009, Tweedie et al. 2007).

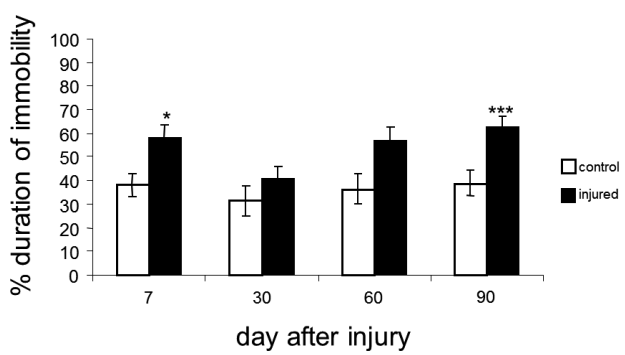

Fig. 4. Forced swimming test, % duration of immobility. Mice, when placed into the cylinders for the first time, swim vigorously around apparently searching for an exit. After 2 min, the percentage of time spent in immobility was measured, and a difference between the two groups was found using a two-way ANOVA ($p<0.001$) and also a statistical influence of the test day ($p<0.05$) (i.e., the sham-injured group was immobile for less time than the injured group). There is no interaction between group and test day ($p>0.05$). Significance after Bonferroni's multiple comparison tests: *$p<0.05$, **$p<0.01$.

Interestingly, the learning deficits shown in most tests we used namely; the MWM, the swim T-maze and the memory associated passive avoidance test (Zohar et al. 2003, Milman et al. 2005), all started 30 days after the injury. This delay in learning deficits further implicates apoptosis and other secondary damage to the injured brain, which culminates into long-term cognitive deficits.

Our results also demonstrate clear behavioral abnormality in the forced swim test preformed by the injured mice. Following injury, the mice were immobile for significantly longer periods than control mice. Since immobility was suggested to reflect a state of depressive mood in mice (Smith et al. 1998), our results suggest that mice suffering from mTBI tend to develop a depressive-like behavior starting from 3 days after the injury. These results suggest that mTBI cause an early-onset of depressive-like behavior in the mice, as opposed to the late-onset of the learning and memory deficits.

Our closed-head mTBI model in mice demonstrates an early emergence of depressive-like behavior and persistent learning deficits that are similar to human post concussion syndrome**.** This suggests that our model can serve as a useful tool for the study of the complex relationship between cognitive/behavioral alterations and corresponding neuronal damage occurring after a minor to mild head trauma. This model may prove useful in the development of novel therapeutic approaches.

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