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Investigation of Behavioural and Neuropathological Alterations Caused by Repetitive Mild Traumatic Brain Injury in a Rodent Model

PhD Thesis

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PhD

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INTRODUCTION

Traumatic brain injury is a very heterogeneous disease and occurs when an external physical force impacts the head, either causing the brain to move within the intact skull or damaging the brain by fracturing the skull (McGinn and Povlishock 2016). Based on the level of consciousness and responsiveness following injury, TBI has been classified into mild, moderate and severe injuries, using Glasgow Coma Scale (GCS) score (Sternbach 2000). Cognitive outcome following head-on collision TBI very much resemble the memory deficits reported in patients following frontal lobe damage, e.g., memory loss, impulsivity and emotional instability (Vakil 2005). However, as stated earlier, concussions or mild TBI (mTBI) are much more common and more frequently observed. Concussions are a frequent occurrence in contact sports such as football, soccer, rugby and hockey. Two primary complications of concussion are the post concussion syndrome and the second impact syndrome (Cantu 1998; Rabadi and Jordan 2001). Increasing evidence has suggested that athletes may sustain multiple concussions throughout their active career, thus potentially exacerbating their cognitive functions. Understanding the long-term sequelae of concussion in humans has been challenging for investigators due to the range of severity of injury, heterogeneous nature of outcome, and feasibility of extended follow-up. In the recent decade, there has been a growing interest to investigate the neuropsychological and pathological effects of repetitive concussion in experimental animal models. Most TBI studies have been primarily conducted in rodents, as they offer the ability to investigate molecular and neurophysiological changes from minutes to weeks following the injury. For example, adult mice who received 3 mild impacts with an inter-injury interval of 24h exhibited significant deficits in learning in the Morris water maze (MWM) test 1 week post-injury, compared to mice who received a single hit (Nichols et al. 2016). Extensive axonal damage caused by repetitive mTBI has also been reported elsewhere (McAteer et al. 2016; Mouzon et al. 2012; Ojo et al. 2016). Heightened tauopathy and glial activation markers such as glial fibrillary acidic protein (GFAP) have also been observed to occur following repetitive mTBI (rmTBI) (Rubenstein et al. 2019; Shitaka et al. 2011). To sum up, most of the currently available studies have only investigated acute and sub-acute effects (at 1 week to 2 weeks postinjury) of TBI and research has paid little or no attention to long-lasting functional consequences of head injuries.

AIMS

Therefore, the general aim of the present series of investigations was to design an mTBI animal model in rats, study the short and long-term behavioural effects of mTBI in normal rats and rats with hypertension as a frequent comorbidity of TBI, and subsequently investigate and improve behavioural effects of repetitive mTBI in rats.

Specific aims:

Study 1: To develop a mTBI model in adult rats, which would cause only short but not prolonged effects on cognition and memory, by testing TBI of different severities and survival intervals.

Study 2: To test the sub-acute behavioural effects of mTBI in normotensive and hypertensive rats.

Study 3: To develop an rmTBI model in adult rats, and asses the short-term and long-term behavioural and pathological effects. To best mimic the outcome of different human repetitive head trauma scenarios, we aim to develop two rmTBI models with short and long inter-injury intervals.

Study 4: To alleviate the cognitive deficits expected in an rmTBI model with memantine, a glutamtatergic NMDAR-receptor antagonist, at sub-acute and chronic stages of TBI.

METHODS

Experimental traumatic brain injury

Animals were anaesthetised with isoflurane gas. Anaesthesia was induced for 5 min with 4% isoflurane (Forane, Abbott, Hungary) in 70% N_2O and 30% O_2 in an induction box, and maintained under anaesthesia throughout the injury and surgical procedure. Rats were then ventilated with 1.5% isoflurane in 70% N_2O and 30% O_2 (Inspira ASV, Harvard Apparatus USA). Once the anaesthesia was stabilized, the animals were exposed to an impact acceleration TBI procedure initially described for rats by Foda and Marmarou (1994). A midline incision was made on the skin to expose the skull. A stainless-steel disc (10 mm in diameter and 3 mm thickness) was fixed on the skull in the sagittal midline, centrally between the lambda and bregma landmarks using cyanoacrylate adhesive, in order to reduce the risk of skull fracture. The rat was placed prone on a foam bed under a 2 m high hollow Plexiglass tube with an inner

diameter of 10mm, which contained 9 cylindrical brass weights, weighing 50 g each which were attached to each other. The 450 g weight was dropped onto the stainless disc fixed to the rat's skull. Severity of injury was determined as the height from which the weight was dropped. The exposed scalp was sutured, and the rat was placed in an empty cage for recovery. Sham animals were prepared for injury in the same fashion, but were not injured. For study 3, repetitive mTBI with 24 hr inter-injury interval were operated and anesthetized to receive one 15cm injury on each day, for five days, whereas those with shorter inter-injury interval received all the five injuries on the same day, under a single, continuous administration of anaesthesia.

Open Field Test

Locomotor activity was measured in the open field test (OFT) apparatus. Open field test sessions were run on the day before the novel object recognition (NOR) test sessions (see below) in order to habituate the rats to the arena. The OFT was performed in an open field box which was made of black-coloured plywood, in size of 57.5x57.5 cm (length x width) surrounded by 39.5 cm high walls. The floor of the arena was divided with light grey painted lines to four by four equal squares. The four squares in the middle of the arena, which were not bordered by walls, were considered together as the centre area of the arena. In each session, rats were allowed to explore the OFT arena for 5 min. After each session, the box was thoroughly cleaned using 20 v/v % ethanol. Line crossings were counted manually. All animals were tested for baseline measurements (pre-injury) and at post-injury.

Novel Object Recognition Test

The NOR test included 2 trials – one acquisition trial followed by one retention trial after a certain delay (Ennaceur and Delacour 1988). In the first (acquisition) trial, the rat explored 2 identical objects (familiar, f + f) placed in the arena for a duration of 3 min. After a delay, a second (recognition) trial was run with one object identical to the sample and a novel object (n) introduced, which had never been seen by the animal before (f + n). Observation behaviour of the animals in the second trial was also recorded for 3 min. During the delay period, rats were not transferred back to the animal house; they were kept in an empty cage, in a dark room located next to the testing room. In both trials, the time spent with the exploration of one or the other

objects was recorded. The animal was considered to explore a given object, when it sniffed the object or put his nose close to it while facing the object. Object-pairs were distributed randomly between animals and experimental sessions in a counterbalanced latin-square design. In the first trial of each NOR test, overall exploratory activity was measured by summing the exploration times for the two objects (SumE1). In the second trial, the time spent with the exploration of the novel (En) and the familiar (Ef) objects were compared by calculating a discrimination index (DI) using the following equation:

$$DI = (En - Ef) / (En + Ef).$$

Rats with low exploratory drive in the second trial (i.e., did not observe the two objects together for at least 5 s), or with +1.00 or −1.00 DI were excluded from the analysis.

Morris Water Maze Test

Short- and long-term spatial memory of the rats was tested in the Morris water maze (MWM) using a blue, circular pool, 180 cm in diameter and 90 cm in height (Ugo Basile, Gemonio, Italy). The floor area of the pool was divided into four virtual quadrants (NW, SW, SE, NE). The rats were trained in the water maze task in four training sessions on four consecutive days with four trials for each animal on each day. On training days, a hidden platform was placed in the centre of the SW quadrant. In each trial, rats were put into the water facing the wall of the NW quadrant at the beginning of the session, and then in the following trials in a clockwise direction, and were allowed to search for the hidden platform for 120 s. The time elapsed until finding the platform (i.e., sitting on it) was measured as escape latency. If the rat failed to find the platform after 120 s, it was gently guided and transferred to the platform by the experimenter, and the cut-off time was recorded as escape latency. On the fifth day, the platform was removed from the pool. A single probe trial was performed, and rats were allowed to explore the pool for 120 s. The time spent in the target quadrant was measured during the probe trial as a readout of long-term memory.

Enzyme-Linked Immuno Sorbent Assay

For study 3, blood serum samples were collected from each group. Commercially available sandwich Enzyme-Linked Immuno Sorbent Assay (ELISA) kits

(Elabscience®, USA) were used to measure concentration of serum pTau protein (cat. no. E-EL-R1090), GFAP (cat. no. E-EL-R1428) and S100β protein (cat. no. E-EL-R0868). Protein concentrations in different experimental groups measured with ELISA were compared using Kruskal-Wallis non-parametric rank test and Dunn's post-hoc test.

Immunohistochemistry

For Study 3, brains of rats from each experimental group were removed and immersed in the same fixative overnight (16–18 h). A midline, 5mm-wide block of the brainstem was removed using a sagittal brain blocking device (Acrylic Brain Matrix for Rat, World Precision Instruments, Sarasota, FL) to include the region extending from the interpeduncular fossa to the second cervical segment. All blocks were sectioned sagittally with a Vibratome Series 1500 Tissue Sectioning System (Technical Products International Inc., St. Louis, MO) at a thickness of 40 μm and collected in PBS. Sections were collected in a serial fashion then processed for immunohistochemical localization of damaged axonal profiles via the detection of the amyloid precursor protein (APP).

RESULTS

Study 1: Behavioural Effects of Traumatic Brain Injury of Different Severities

For this study, four experimental groups were used: Sham (no injury), Mild1 (15 cm injury), Mild2 (25 cm injury) and Severe (Seve, 150 cm injury). In the OFT, animals of all the injury groups exhibited overall good locomotor function in the pre-injury test, with no statistical difference in performance ($F(3,44) = 1.180$; $p=0.328$). All the injury groups performed similarly in both the post-injury 1 week ($F(3,44) =1.074$; $p=0.370$), post-injury 4 weeks ($F(3,44) = 0.307$; $p = 0.820$, and the post-injury 8 weeks tests ($F(3,44) = 0.116$; $p=0.950$), indicating no major impairment in locomotor function as a result of any types of TBI.

In the pre-injury NOR test, all groups were able to discriminate between the familiar and the novel object. Discrimination index value for each group was above the chance level. However, in the post-injury 4 weeks NOR session, only the Sham group was able to discriminate between the familiar and the novel object, while Mild1, Mild2

and Seve groups did not recover. In the post-injury 8 week session, Mild1 injury group, along with Sham, recovered and was able to perform above chance level. Mild2 and Seve still did not discriminate the familiar and novel objects (See Fig. 1). In the MWM, at post-injury 3 weeks, mixed-ANOVA for escape latency indicated that there was no interaction effect between injury groups and training days. Marginally significant between-subject effect was found in the probe trial timing, and Severe group was found to perform the worst, while Sham, Mild1 and Mild2 performed similarly (Kruskal-Wallis: $p=0.09$; Mann-Whitney: Mild1-Seve, Mild2-Seve $p<0.05$). To sum up, mTBI evoked from a height of 15 cm caused no significant long-term neurocognitive alterations. We also found that the 25 cm injury did not appear to be significantly different in the NOR test from the 15 cm injury group. From the results we concluded that the 15 cm injury may be suitable to be utilised as a base to develop repetitive mTBI models in rodents in order to replicate and understand the impact and the effects of repetitive concussive injuries in humans.

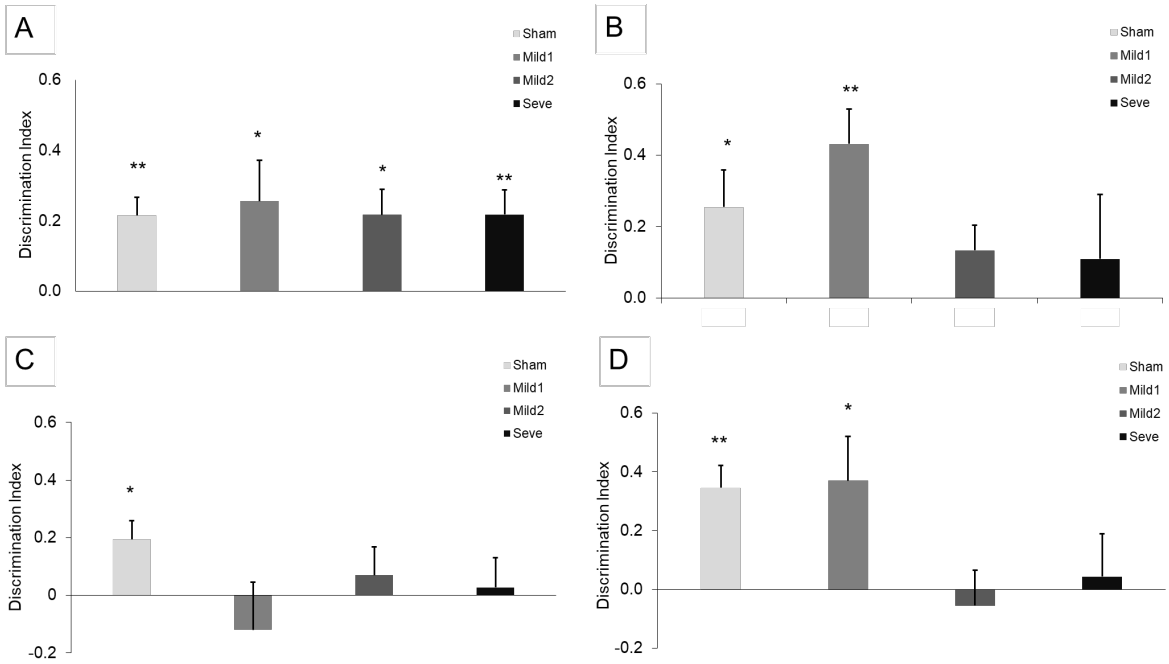

Figure 1: Result of novel object recognition test (NOR). Insets depict measurement point at Pre-injury (A), post-injury 1 week (B), post-injury 4 weeks (C), and post-injury 8 weeks (D). Abbreviations: one-sample t-test $p<0.05$; **one-sample t-test $p<0.01$.*

Study 2: Behavioural Effect of Mild Traumatic Brain Injury in Hypertensive Rats

Wistar-normotensive rats, and spontaneously hypertensive rats (SHR) underwent a single 25 cm TBI treatment. In the pre-injury OFT session, both groups showed similar locomotor activity, indicated by the number of line crossings. In the post-injury 2 weeks session, Wistar rats showed significantly less number of line crossings, which could indicate habituation while SHR rats did not. In the pre-injury NOR session, SHR rats significantly discriminated the objects ($p<0.01$), while the normotensive Wistar rats did not ($p=0.424$). At the two weeks post-injury NOR session, control normotensive Wistar rats behaved similar to the pre-injury session (0.388 ± 0.165; $t=2.310$, $df=4$, $p=0.081$). However, mTBI resulted in a significant ($p<0.05$) decrease in the DI of SHR rats indicating impaired memory function (SHR-TBI main effect: $F(1, 22)=5.223$, $p<0.05$)). Discrimination in the SHR+mTBI group was significantly worse than that of the Wistar+mTBI and SHR rats ($p<0.05$) (See Fig. 2). In conclusion, pre-existing hypertension in rats exacerbates the behavioural outcome caused by mTBI.

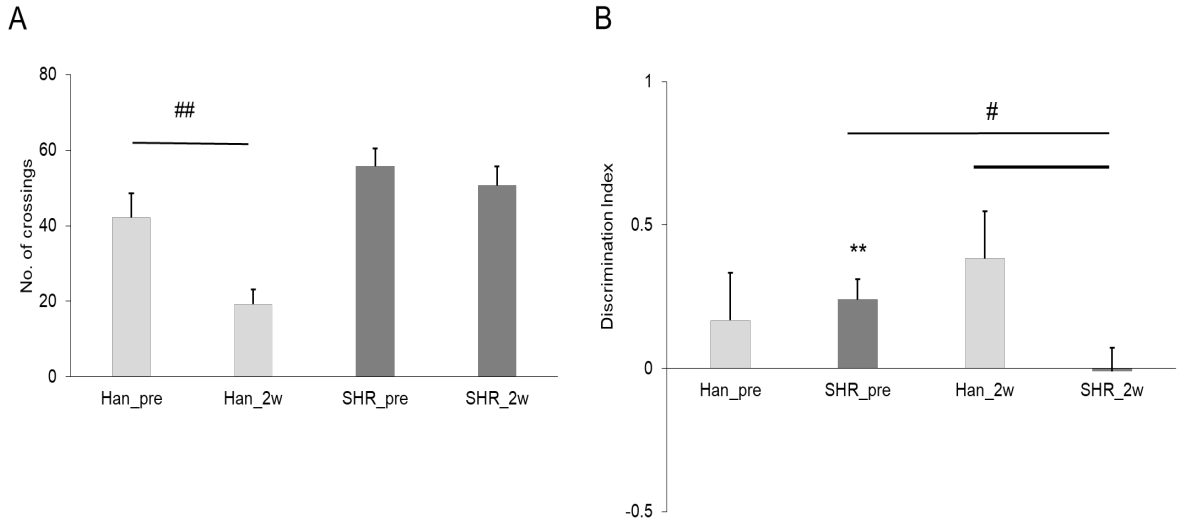

*Figure 2. Locomotor activity and working memory were tested with OFT and NOR respectively. (A) In the OFT, compared to pre-injury session, only Wistar+mTBI (n=14) group showed significantly less number of line crossings at post-injury 2 weeks ($p<0.01$). (B) In the pre-injury session, SHR rats were able to significantly discriminate the objects. Wistar+mTBI (n=5) rats performed above chance level in the post-injury 2 weeks session, while SHR+mTBI (n=11) rats were significantly worse than Wistar+mTBI rats and pre-injury SHR rats ($p<0.05$). Abbreviations: ** one-sample t-test $p<0.01$; # Tukey's pairwise comparison $p<0.05$; ## Tukey's pairwise comparison $p<0.01$.*

Study 3: Behavioural and Molecular Effect of Repetitive Mild TBI

Five experimental groups were used: Sham (no injury), mTBI (single 15cm hit), rmTBI (five 15 cm hits, 24 hours apart), rapTBI (five 15 cm hits, 5 minutes apart), and sTBI (single 15o cm hit). In the OFT sessions, no significant difference was observed in number of line crossings between the groups, pre- and post-injury. In the NOR test, while in the pre-injury, all groups performed similarly, in the post-injury 2 weeks test, both repetitive injury groups were unable to discriminate between the familiar and the novel objects. In the post-injury 8 weeks session, rmTBI group still failed to discriminate between the novel and the familiar object, and was worse compared to Sham, mTBI and rapTBI groups. In the MWM post-injury 3 weeks test, there was a significant decrease of escape latency in all groups during the training days. However, in the probe trial timings, compared to Sham and single mTBI groups, only rapTBI group performed significantly worse (Fig. 3).

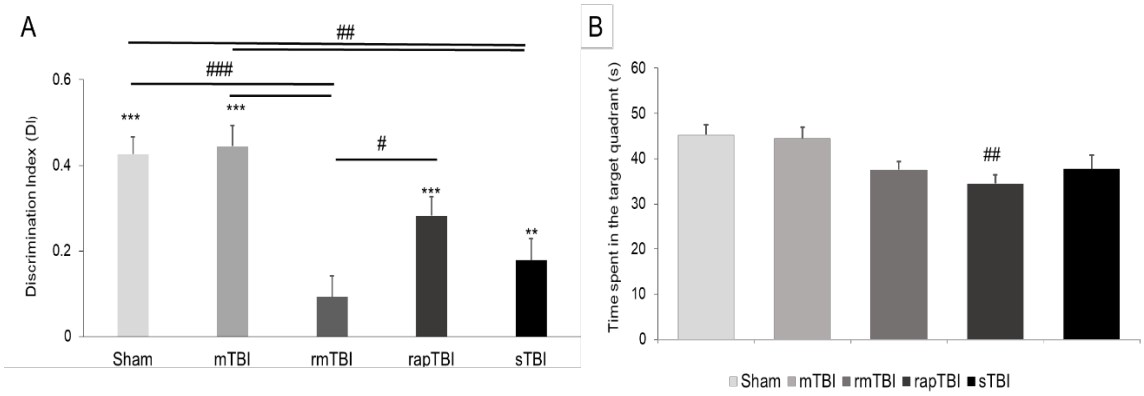

*Figure 3: Repetitive mild TBI worsen the overall behavioural outcome of the injury, as seen in NOR and MWM tests. (A) At post-injury 8 weeks session, the rmTBI group had significantly worse DI than the Sham, mTBI and rapTBI. (B) in the MWM post-injury 3 weeks probe trial timings, the rapTBI group performed significantly worse than the Sham and mTBI groups. Abbreviations: ** one-sample t-test p<0.01; *** one-sample t-test p<0.001; # Tukey's pairwise comparison p<0.05; ## Tukey's pairwise comparison p<0.01; ### Tukey's pairwise comparison p<0.001.*

Eight weeks following injury, ELISA test showed that only sTBI group had elevated GFAP levels, compared to Sham, mTBI and rapTBI groups. Serum levels of s100B and pTau were not statistically different in the injury groups. Finally, at post-injury 24 hours, only a few, scattered APP immunopositives were observed in the sTBI group. From our findings, compared to the rapTBI injury, the rmTBI scenario, with

24 h inter-injury interval, displayed long-term cognitive deficits without histological consequences.

Study 4: Pharmacological Amelioration of Cognitive Deficits Caused by Repetitive Mild Traumatic Brain Injury

For this study, memantine, an NMDA receptor antagonist was administered at sub-acute and chronic stage following rmTBI, at 0.1mg/kg, 0.3mg/kg and 1.0mg/kg doses. For the NOR post-injury 6-8 weeks sessions, memantine treatment was administered in a within-subject design: memantine doses were counterbalanced with NOR sessions. Sham and rmTBI-control groups were used as negative and positive control respectively. Results showed that, in the NOR post-injury 6-9 weeks session, the Sham group could discriminate between the novel and the familiar objects, while rmTBI-control failed to do so. In the post-injury memantine NOR session, only Mem1.0 treatment ($p<0.01$) improved the performance of the rats to discriminate between familiar and novel objects (Fig. 4).

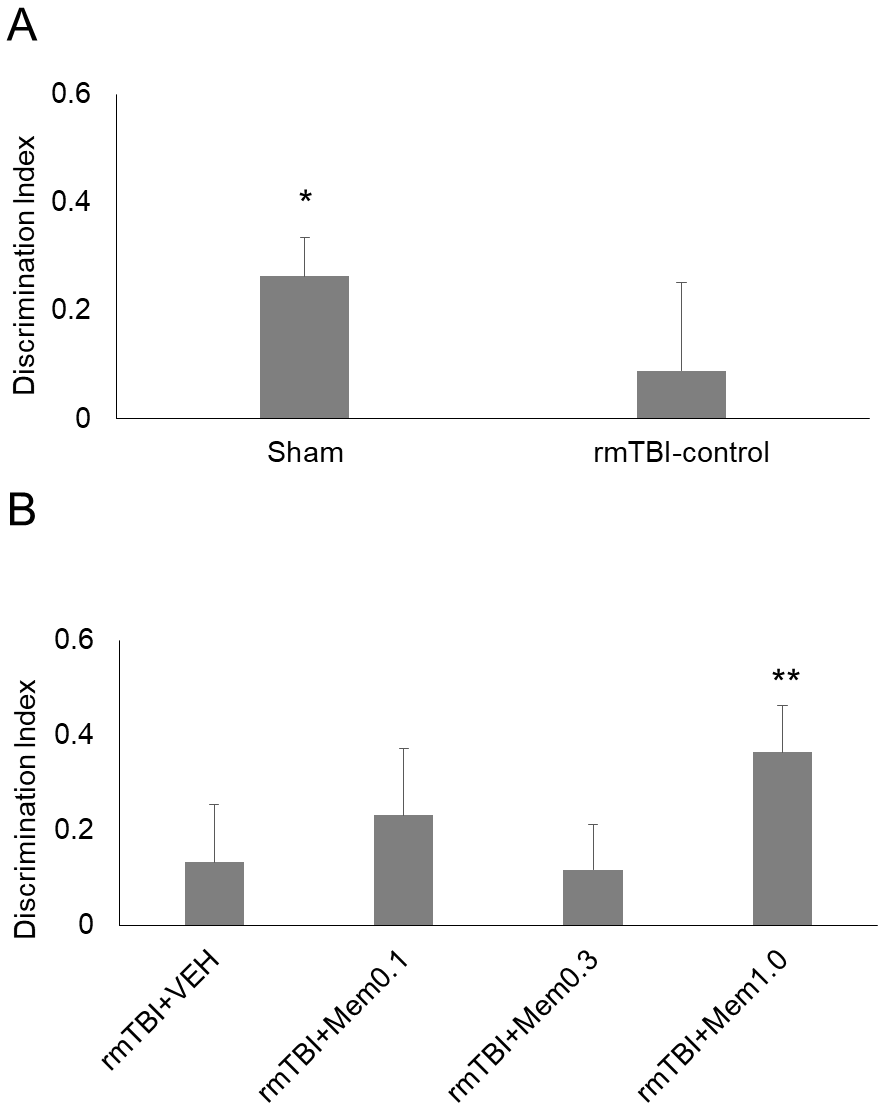

*Figure 4: Effects of memantine in post-injury 6-9 weeks NOR sessions. (A) The rmTBI-control group could not discriminate between familiar and novel objects, while the Sham control group could (Sham: n=6, p<0.05; rmTBI-control: n=7, p=0.608). (B) In the within-memantine session, only the highest memantine dose (rmTBI+Mem1.0) could improve the performance of the rmTBI injured rats to discriminate between the familiar and novel objects. Abbreviations: * one-sample t-test p<0.05; ** one-sample t-test p<0.01.*

For the MWM session, at post-injury 3 weeks, a between-subject design was used, to avoid habituation to the task apparatus in repeated sessions. Forty minutes before the first trial on each acquisition day, rmTBI rats received either memantine (Mem0.1/Mem0.3/Mem1.0) or no treatment. The Sham group served as control. For the acquisition phase, escape latency data indicated that there was no interaction effect

between treatment with memantine and training days, i.e., treatment with memantine did not reduce escape latency on any of the training days, compared to the control groups. In the probe trial, treatment with memantine did not improve reference memory in recalling the location of the target quadrant in the TBI groups compared to the rmTBI-control group. From the results, we found that high dose of memantine was successful in attenuating working memory deficits in NOR test. Memantine at 1.0mg/kg dose was able to improve the discrimination index above the chance level, while vehicle treatment could not discriminate significantly. However, treatment with memantine did not significantly improve spatial working memory and retention in the MWM test, compared to sham-injured and rmTBI-control (rmTBI-control) groups.

SUMMARY AND CONCLUSIONS

Mild traumatic brain injury (mTBI) is most often associated with short-term cognitive dysfunction that tends to resolve within three months of injury. Not only does pre-existing comorbid conditions, such as hypertension, increase risk of mortality in TBI, repetitive mTBIs may increase the risk of developing neurodegenerative disorders, such as dementia, in old age. Therapeutic intervention to treat TBI-related cognitive deficits is an unmet medical need.

Using the Marmarou impact acceleration model, two single hit mild TBI models were designed: TBI was induced from the height of 15 or 25 cm. Furthermore, we tested the 25 cm injury in spontaneously hypertensive rats to assess the behavioural outcome. We further utilised the 15 cm injury model to design two repetitive mild TBI (rapTBI, rmTBI) models, with short and long inter-injury intervals, respectively. Finally, in order to treat rmTBI-induced cognitive impairment, different doses of the NMDAR antagonist memantine, were given at sub-acute and chronic stages of the injury. Novel object recognition (NOR), and Morris water maze (MWM) tests were used to assess behavioural outcome.

We found that mild TBI evoked from a height of 15 cm caused no significant long-term neurocognitive alterations. Animals with acute deficits observed following mTBI in the behavioural tasks fully recovered by two months after the injury, also showing no remaining gross changes in the integrity of the corpus callosum. In addition, we

also found that the 25 cm injury did not appear to be significantly different in the NOR test from the results of the 15 cm injury.

Working memory deficits two weeks following a 25 cm TBI treatment were observed in SHR and control normotensive rats at two weeks following a 25 cm TBI treatment. Hypertension-associated pathologic changes in the brain most likely exacerbates the TBI-induced excitotoxicity (Raz, Rodrigue, and Acker 2003; Szarka et al. 2017), causing long-term cognitive dysfunction, which could explain the deficits in the NOR test at sub-acute phase.

In repetitive mild TBI, the interval between the successive injuries plays a critical role in determining the extent and persistence of cognitive impairment. We found that, compared to sham-injured and single mTBI animals, rmTBI evoked from a height of 15 cm, with 24h inter-injury interval, caused persistent neurocognitive alterations at 8 weeks following the last injury event. It has been suggested that successive impacts, most likely, lead to dysfunction of auto regulation of intracranial and cerebral perfusion pressures, causing heightened damage than a single impact (McCrory and Berkovic 1998). In line with that, we also showed that the rmTBI protocol serves as an efficient model to study the outcome of multiple concussions.

Finally, glutamatergic NMDA receptor antagonist memantine at 1.0 mg/kg dose was efficient in reversing working memory deficits in the NOR test 6-9 weeks following repetitive injury. Results of several preclinical TBI studies indicate that glutamate-mediated excitotoxicity plays an early and critical role in the cascade of secondary injury events following TBI (Katayama et al. 1990; Palmer et al. 1993; Takahashi, Manaka, and Sano 1981; Yi and Hazell 2006). In line with the notions above, in the present experiment, memantine appeared to be effective in putatively attenuating NMDAR overactivity even several weeks after the injury. We conclude that, based on our present findings, rmTBI causes long-term behavioural deficits, with no gross axonal injury, which could be reversed with treatment with memantine.

To our knowledge, this is the first study to address targeting NMDAR at sub-acute and chronic stage, after a repetitive (5-hit) mild (15-cm) TBI in rats. It is possible that the three-week period of time elapsed before the administration of memantine may be rather late after the initial insult and an additional pharmacological intervention administered closer to the time of injury may be likely to produce a more robust neuroprotective effect. Further research is necessary to find effective targets, as well

as pharmacological agents, to treat and reverse most, if not all, of the cascade of metabolic events that occur following TBI.

PUBLICATIONS

List of peer-reviewed journal articles related to thesis

Tadepalli SA, Bali ZK, Bruszt N, Nagy LV, Amrein K, Fazekas B, Büki A, Czeiter E, Hernádi I. (2020) *Long-term cognitive impairment without diffuse axonal injury following repetitive mild traumatic brain injury in rats.* Behavioural Brain Res. 378:112268. doi: 10.1016/j.bbr.2019.112268

Impact Factor: 2.77

Szarka, N, Toth, L, Czigler, A, Kellermayer, Z, Ungvari, Z, Amrein, K, Czeiter, E, Bali, ZK, Tadepalli, SA, Wahr, M, Hernadi, I, Koller, A, Buki, A, Toth, P. (2019) *Single mild Traumatic Brain Injury Induces Persistent Disruption of the Blood-Brain Barrier, Neuroinflammation and Cognitive Decline in Hypertensive Rats.* Int J Mol Sci 20(13):3223. doi: 10.3390/ijms20133223

Impact Factor: 4.183

List of other peer-reviewed journal articles

Bali ZK, Bruszt N, Tadepalli SA, Csurgyók R, Nagy LV, Tompa M, Hernádi I. (2019) *Cognitive enhancer effects of low memantine doses are facilitated by an alpha7 nicotinic acetylcholine receptor agonist in scopolamine-induced amnesia in rats.* Front Pharmacol 10:73. doi: 10.3389/fphar.2019.0007

Impact Factor: 3.8

List of conference abstracts related to thesis

Tadepalli S. A., Bruszt N., Nagy L. V., Bali Zs. K, Czeiter E., Amrein K, Büki A., Hernádi I. (2019) Repetitive mild traumatic brain injury causes long-term cognitive impairment in rats. Conference of European Behavioural Brain Society, 2019, Prague, Czech Republic

Tadepalli S. A., Bruszt N., Nagy L. V., Bali Zs. K, Czeiter E., Amrein K, Büki A., Hernádi I. (2019) *Repetitive mild traumatic brain injury causes long-term cognitive impairment in rats.* Conference of The Hungarian Neuroscience Society, 2019, Debrecen, Hungary

Tadepalli S. A., Bruszt N., Nagy L. V., Bali Zs. K, Czeiter E., Amrein K, Büki A., Hernádi I. (2018) *Repetitive mild traumatic brain injury causes long-term cognitive impairment in rats.* FENS, 2018, Berlin, Germany

Tadepalli S. A., Bruszt N., Nagy L. V., Bali Zs. K, Czeiter E., Amrein K, Buki A., Hernádi I. (2018) *Repetitive mild traumatic brain injury causes long-term cognitive impairment in rats.* EMN, 2018, Pecs, Hungary.

Tadepalli S. A., Bali Zs. K., Bruszt N., Czeiter E., Amrein K., Vranesics A., Berente Z., Büki A., Hernádi I. (2017) *Evaluation of Cognitive Dysfunction and White Matter Integrity following Mild Traumatic Brain Injury in Rats.* CNS Symposium, 2017, Pécs, Hungary.

REFERENCES

Cantu, R C. 1998. "Second-Impact Syndrome." *Clinics in Sports Medicine* 17: 37–44.

Ennaceur, A, and J Delacour. 1988. "A New One-Trial Test for Neurobiological Studies of Memory in Rats. 1: Behavioral Data." *Behavioural brain research* 31(1): 47–59.

Katayama, Y., D. P. Becker, T. Tamura, and D. A. Hovda. 1990. "Massive Increases in Extracellular Potassium and the Indiscriminate Release of Glutamate Following Concussive Brain Injury." *Journal of Neurosurgery* 73(6): 889–900.

McAteer, K M. et al. 2016. "Short and Long Term Behavioral and Pathological Changes in a Novel Rodent Model of Repetitive Mild Traumatic Brain Injury." *PLoS ONE* 11(8).

McCrory, Paul R, and Samuel F Berkovic. 1998. “Second Impact Syndrome.” *Neurology* 50(3): 677 LP – 683. http://n.neurology.org/content/50/3/677.abstract.

McGinn, Melissa J., and John T. Povlishock. 2016. “Pathophysiology of Traumatic Brain Injury.” *Neurosurgery Clinics of North America* 27(4): 397–407.

Mouzon et al. 2012. “Repetitive Mild Traumatic Brain Injury in a Mouse Model Produces Learning and Memory Deficits Accompanied by Histological Changes.” *Journal of Neurotrauma* 29(18): 2761–73.

Nichols, J N. et al. 2016. “Greater Neurobehavioral Deficits Occur in Adult Mice after Repeated , as Compared to Single , Mild Traumatic Brain Injury (MTBI).” *Behavioural Brain Research* 298: 111–24.

Ojo, J O. et al. 2016. “Chronic Repetitive Mild Traumatic Brain Injury Results in Reduced Cerebral Blood Flow, Axonal Injury, Gliosis, and Increased T-Tau and Tau Oligomers.” *Journal of Neuropathology and Experimental Neurology* 75: 636–55.

Palmer, A M. et al. 1993. “Traumatic Brain Injury-Induced Excitotoxicity Assessed in a Controlled Cortical Impact Model.” *Journal of Neurochemistry* 61(6): 2015–24.

Rabadi, M H., and B D. Jordan. 2001. “The Cumulative Effect of Repetitive Concussion in Sports.” *Clinical Journal of Sport Medicine* 11: 194–98.

Raz, N., K M. Rodrigue, and J D. Acker. 2003. “Hypertension and the Brain: Vulnerability of the Prefrontal Regions and Executive Functions.” *Behavioral Neuroscience* 117: 1169–80.

Rubenstein, . et al. 2019. “Novel Mouse Tauopathy Model for Repetitive Mild Traumatic Brain Injury: Evaluation of Long-Term Effects on Cognition and Biomarker Levels after Therapeutic Inhibition of Tau Phosphorylation.” *Frontiers in Neurology* 10: e-collection.

Shitaka, Y. et al. 2011. “Repetitive Closed-Skull Traumatic Brain Injury in Mice Causes Persistent Multifocal Axonal Injury and Microglial Reactivity.” *Journal of Neuropathology and Experimental Neurology* 70: 551–67.

Sternbach, G L. 2000. “The Glasgow Coma Scale.” *Journal of Emergency Medicine* 19: 67–71.

Szarka, N. et al. 2017. “Hypertension-Induced Enhanced Myogenic Constriction of Cerebral Arteries Is Preserved after Traumatic Brain Injury.” *Journal of Neurotrauma* 34: 2315–19.

Takahashi, H., S. Manaka, and K. Sano. 1981. “Changes in Extracellular Potassium Concentration in Cortex and Brain Stem during the Acute Phase of Experimental Closed Head Injury.” *Journal of Neurosurgery* 55(5): 708–17.

Vakil, E. 2005. “The Effect of Moderate to Severe Traumatic Brain Injury (TBI) on Different Aspects of Memory: A Selective Review.” *Journal of Clinical and Experimental Neuropsychology* 27(8): 977–1021.

Yi, J H., and A S. Hazell. 2006. “Excitotoxic Mechanisms and the Role of Astrocytic Glutamate Transporters in Traumatic Brain Injury.” *Neurochemistry International* 48: 394–403.