LONG-TERM EFFECTS OF TRAUMATIC BRAIN INJURY ON EMOTION AND COGNITION IN ATHYMIC NUDE RATS

Luci López- Velázquez, Daniel L Haus, George A Lacuesta, Janae Bustos, Harvey Perez, Eric Gold, Diane Su, Aileen J Anderson & Brian J Cummings

Sue & Bill Gross Stem Cell Research Center, University of California, Irvine 2030 Gross Hall, CA 92697-1705, USA

Introduction

Traumatic Brain Injury (TBI) is an alteration in brain function caused by an external force. An estimated of 1.7 million head injuries occur every year in the United States. Around 40% of TBI patients suffer long-term disabilities in cognition, emotion, sensation and movement. Following initial TBI, secondary brain injury progress for days and weeks, thus offering a window of opportunity for therapeutic interventions, and the stem cell therapy is an alternative for neuronal repair and functional recovery. Nevertheless, preclinical testing depends on the selection and characterization of appropriate animal models. We suggest that 2 months post-TBI is the minimum period needed to evaluate human cell transplant efficacy and safety. A 2 month survival and assessment period would allow sufficient time for differentiation and integration of human neural stem cells with the host.
However, few papers have studied functional outcome at a minimum of 2 months post-TBI. We reviewed published TBI literature and we found that only 10% of papers evaluated functional outcome ≥2 months post-TBI and only 8.6% showed functional deficits ≥2 months post-TBI.
The aim of the present study was, to evaluate long-term deficits on emotional and cognitive behaviors in a Controlled Cortical Impact (CCI) injury model in Athymic Nude Rats (ATN). ATN rats are immuno-deficient, which gives an opportunity to use human stem cells for transplantation.

Materials and Method

Surgery

Male ATN rats were anesthetized, using a stereotaxic coordinates. A craniotomy of 6 mm diameter was performed using a trephine over the left cortex. Once the craniotomy was performed, CCI was delivered to the left parietal cortex using a 5mm rounded tip, 2.5 mm depth and 4.5m/s velocity and 500 ms duration.

Atlas Paxinos and Watson Coordinates:
A/P -4.5
M/L -3.6

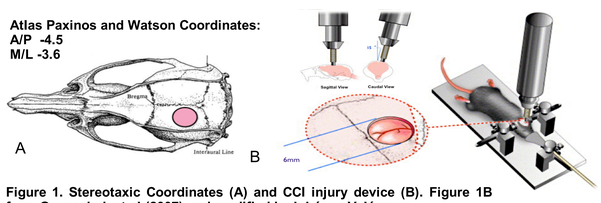

Figure 1. Stereotaxic Coordinates (A) and CCI injury device (B). Figure 1B from Onyszchuk et al (2007) and modified by L López-Velázquez.

Behavior

Novel Place Recognition (NPR) and Novel Object Recognition (NOR)

Nine weeks post-injury, sham and injured rats were trained and tested NPR and NOR.

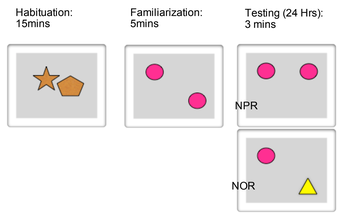

Figure 2. NPR and NOR tests to evaluate recognition memory.

Elevated Plus Maze

Ten weeks post-injury, sham and injured animals were tested on the EPM to evaluate anxiety. Animals were placed in the intersection of the four arms of the elevated plus maze and their behavior was recorded for 5 min.

Figure 3. Elevated Plus Maze

Morris Water Maze (MWM)

Thirteen weeks post-injury animals performed acquisition and reversal of MWM. Animal was placed in four different start positions. MWM assesses spatial learning and memory.

Figure 4. Morris Water Maze

Conditioning Taste Aversion (CTA)

Sixteen weeks post-injury sham and TBI rats were trained in the CTA.

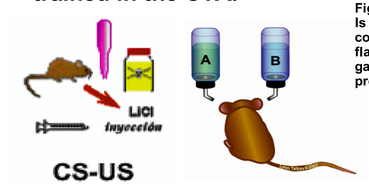

Figure 5. Conditioned Taste Aversion. Is an associative conditioning, which consists in the presentation of a novel flavor (saccharin: CS), followed of gastric malaise (LiCl: US) that provokes aversion to a novel flavor.

Results

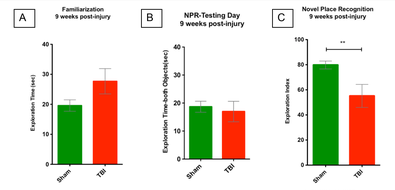

Figure 6. During the familiarization phase (A) and testing day (B & D), not significant differences were found in the total exploration time of both objects (p>0.05). Significant differences were found in the exploration index between injured and sham animals (p<0.01) during NPR testing (C) and NOR testing (E) not significant differences were found (p>0.05). Data represent the mean ± standard error. T Test.

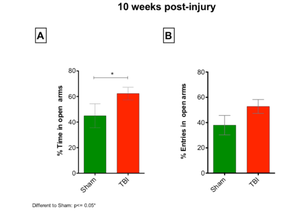

Figure 7. Graphs show percentage of time in open arms (A) and percentage of entries in open arms (B). Injured animals spend more time in open arms than closed arms (p<0.05). Not significant differences were found in percentage of entries (p>0.05). Data represent the mean ± standard error.

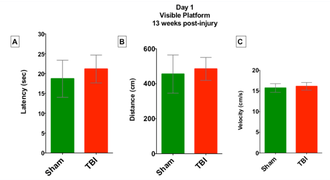

Figure 8. Graphs show latency to find the platform (seconds) (A), distance traveled to find the platform (B) and velocity during the visible platform of MWM (C). No differences were founded (p>0.05). MWM performance in both groups was similar. Data represent the mean ± standard error.

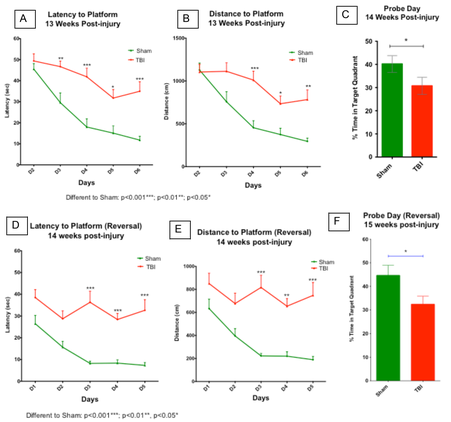

Figure 9. Spatial Acquisition and Reversal of MWM. Latency to find the platform in Spatial Acquisition (A) and Reversal (D). Distance traveled in Spatial Acquisition (B) and Reversal (E). Injured animals spend more time to find the platform (A&D) and travel longer distances (B&E) (p<0.05). During the Probe day (C&F), the performance of Injured rats was significantly different to shams rats (p<0.05). Data represent the mean ± standard error.

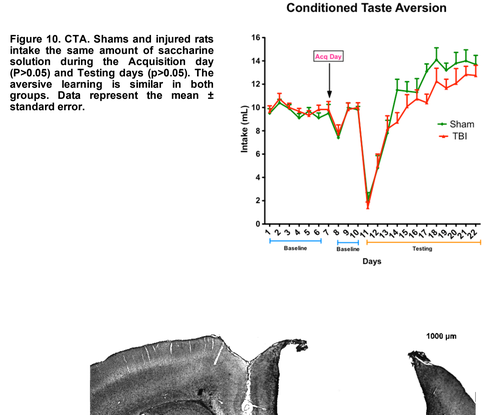

Figure 10. CTA. Shams and injured rats intake the same amount of saccharine solution during the Acquisition day (P>0.05) and Testing days (p>0.05). The aversive learning is similar in both groups. Data represent the mean ± standard error.

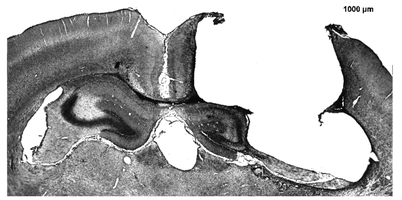

Figure 11. Representative Timm Staining in injured rat brain. Coronal Section, Scale bar: 1000 mm. Magnification 20X.

Conclusion

Novel place recognition was affected in TBI rats. They explored less time in novel than familiar place. Novel object recognition was not affected. TBI rats in EPM spent more time in open arms than closed arms. This behavior suggest less anxiety and more risk behaviors in TBI animals. Injured rats, during MWM showed deficits in spatial acquisition, reversal learning and reference memory. Contextual CTA was not affected in TBI rats.
TBI rats showed deficits in NPR, MWM, EPM after 2 months post-injury, which could indicate that these tasks can be used to evaluate long-term deficits in TBI rats and assess long-term cell transplant efficacy.

Acknowledge

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