EFFECTS OF DEMENTIA ASSOCIATED MUTATIONS IN TREM2 ON TRAUMATIC BRAIN INJURY INFLAMMATION AND PATHOLOGY

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By

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A capstone project submitted for
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University Honors
University of California, Riverside

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Abstract

Traumatic brain injury (TBI) affects 1.7 million people in the United States annually. Previous studies have found that TBI leads to an increased risk of developing Alzheimer's disease. Microglia are the brain resident immune cells that help to regulate neuronal function and have a 10-fold increase in expression of Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) compared to other macrophages. The lack of a functioning TREM2 gene (TREM2KO) leads to early onset cognitive dementia, highlighting its importance. Our model of TBI uses controlled cortical impacts (CCI) to the right hemisphere of C57Bl/6J (WT) and TREM2KO mice in a highly reproducible fashion. These impacts are consistent with a mild TBI, and representative of a concussion. Increased expression of P2Y12, a purinergic receptor expressed on microglia, is a marker of cell damage. Previous data from our TBI model has shown that expression of P2Y12 increases 2-fold in 7 day post TBI TREM2KO mice, when compared to WT. In this study, we do not see a significant increase in 3 day post TBI TREM2KO mice, when compared to WT. However, there is an increase in P2Y12 levels from baseline (no TBI) to 3 day post TBI in both the WT mice (3.44-fold) and TREM2KO mice (4.38-fold). TNFa is another pro-inflammatory marker. We show a significant increase of TNFa in the impacted hemisphere compared to the contralateral hemisphere of 3 day post TBI WT mice.

Acknowledgments

I would like to thank my faculty mentor, Dr. Monica J. Carson, for accepting me into her lab and having confidence in my abilities as a researcher. I am grateful for her guidance and support over the past two years. Additionally, I would like to acknowledge the members of our lab who helped me and worked with me: Yelena Grinberg, Abdullah Madany, Joe Valdez, Juan Tamayo, Geoff Pronovost, and Shaida Abachi. Thank you to the Research in Science and Engineering (RISE) summer research program for sponsoring my research over the 2016 summer. A huge thank you to Amgen for funding this research. Finally, thank you to the University Honors program for fostering a desire to participate in research and complete this capstone project.

Table of Contents

Introduction

Traumatic brain injury (TBI) is an epidemic that affects 1.7 million people in the United States annually. Previous studies have found that TBI leads to an increased risk of developing Alzheimer's disease. Our group studies microglia and its part in mediating neurodegenerative diseases. Microglia are the brain resident immune cells that help to regulate neuronal function and have a 10-fold increase in expression of Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) compared to other macrophages. The lack of a functioning TREM2 gene (TREM2KO) leads to early onset cognitive dementia and heterozygous mutations lead to an increased risk of developing Alzheimer's disease, highlighting its importance in humans. Our model of TBI uses controlled cortical impacts (CCI) to the right hemisphere of C57Bl/6J (WT) and TREM2KO mice in a highly reproducible fashion. These impacts are consistent with a mild TBI, and representative of a concussion. Our lab is interested in studying the effects of dementia associated mutations in TREM2 on TBI inflammation and pathology. We have previously shown increases in P2Y12, a purinergic receptor expressed on microglia, in TREM2KO mice compared to WT mice at 7 days post TBI. This indicates cell damage a week after impact that is more pronounced in mice with TREM2 mutations. Our group is continuing our analysis of inflammatory and pathologic effects of TBI on mice with TREM2 mutations by evaluating mice at 3 days post TBI. TBI visibility has increased significantly in recent years as the sports world, specifically football, has recognized the increased risk of concussions for its participants. There is an increased desire to understand the best course of action for treatment and recovery from TBI, but our knowledge of TBI etiology is lacking. In order to better treat patients with TBI, we must learn more about how the brain is affected in terms of inflammation, pathology, and timeline of the brain response.

Methods

Traumatic Brain Injury Model:

All traumatic brain injury surgeries were performed as controlled cortical impacts (CCI) delivered by an automated, piston-driven impactor tip. The hit was delivered on the right hemisphere of the brain, adjacent to the bregma. The automated nature of the delivery of TBIs allowed our model to have a consistent, reproducible severity of injury across all animals. Our model is consistent with a mild traumatic brain injury, similar to a concussion. [All surgeries completed by Yelena Grinberg].

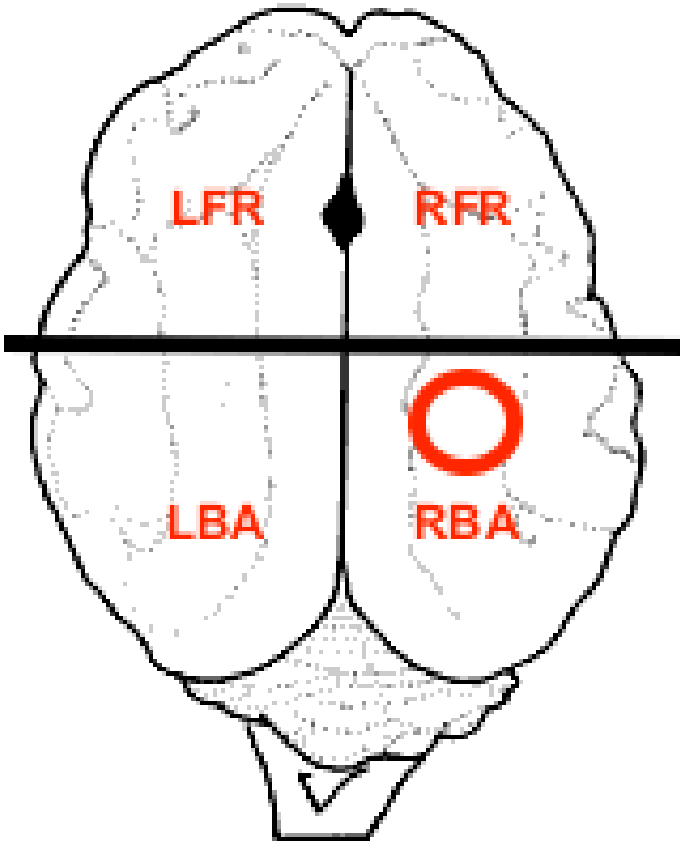


Figure 1: Schematic Model of Traumatic Brain Injury. The schematic demonstrates where the brain regions were separated and the location of the hit. The black line is representative of the division between the frontal bone and the parietal bones (coronal suture). The bregma is the point where the coronal suture and the sagittal suture intersect.

RNA Isolation and cDNA synthesis:

Brains were extracted at the 3 day post TBI time point. They were then separated into 5 separate regions, as shown in the Figure 1. The four labeled regions were from the cortex, and the fifth region (not labeled) was called non-cortical or NC, and included other part of the brain. We took the LBA and RBA regions and completed RNA isolation and cDNA synthesis. [RNA isolations and cDNA synthesis completed by Yelena Grinberg].

Real Time Quantitative Polymerase Chain Reaction (RT-qPCR):

We quantified the amount of mRNA transcripts for various inflammatory markers by using RT-qPCR. Specifically, we use SYBR Green mix to label the transcripts of the gene of interest. In this study, we completed qPCR assays for aquaporin-4 (AQP4), P2Y12, and tumor necrosis factor alpha (TNFa). Primers for the genes of interest are as follows:

AQP4 FWD: AGCCGGCATCCTCTACCTG

AQP4 RVS: CTGCGCGGCTTTGCTGAA

P2Y12 FWD: AGGCTTTGGGAACTTATGC

P2Y12 RVS: GGGTGGTATTGGCTGAGGTG

TNFa FWD: CTGTGAAGGGAATGGGTGTT

TNFa RVS: GGTCACTGTCCCAGCATCTT

HPRT FWD: CCCTCTGGTAGATTGTCGCTTA

HPRT RVS: AGATGCTGTTACTGATAGGAAATCGA

Additionally, a new standard curve was completed with each plate to minimize sources of error. Our TNFa standard curve needed to be extended by two serial dilutions (0.000005 pg/uL and 0.0000005 pg/uL) to capture values with strong resolution. All values were normalized with HPRT transcripts (a common housekeeping gene). Every reported value is a ratio of the number of gene of interest transcripts over the number of HPRT transcripts. [All qPCR assays completed by Samantha Quon].

Results

Analysis of our qPCR data has revealed the following results:

1. We do not see a significant increase in P2Y12 in 3 day post TBI TREM2KO mice, when compared to 3 day post TBI WT mice.

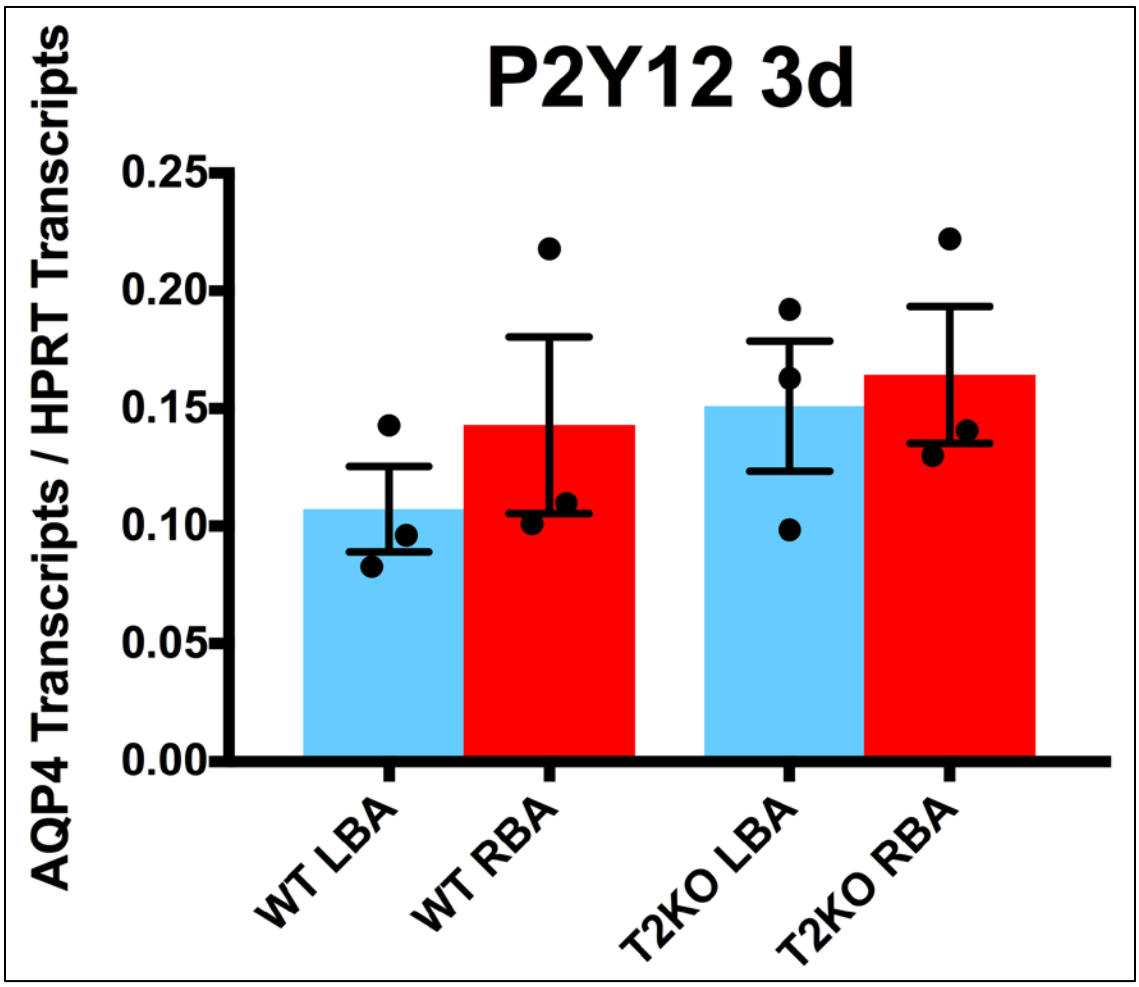


Figure 2: Comparison of WT and TREM2KO expression of P2Y12 at 3 days post TBI. There is no significant difference in the levels of P2Y12 mRNA transcript between the WT and TREM2KO genotypes for the either the impacted hemisphere or the contralateral hemisphere.

2. We see an increase in P2Y12 levels in 3 day post TBI mice, compared to the baseline (no TBI) mice, for the WT genotype (3.44-fold) and TREM2KO genotype (4.38-fold).

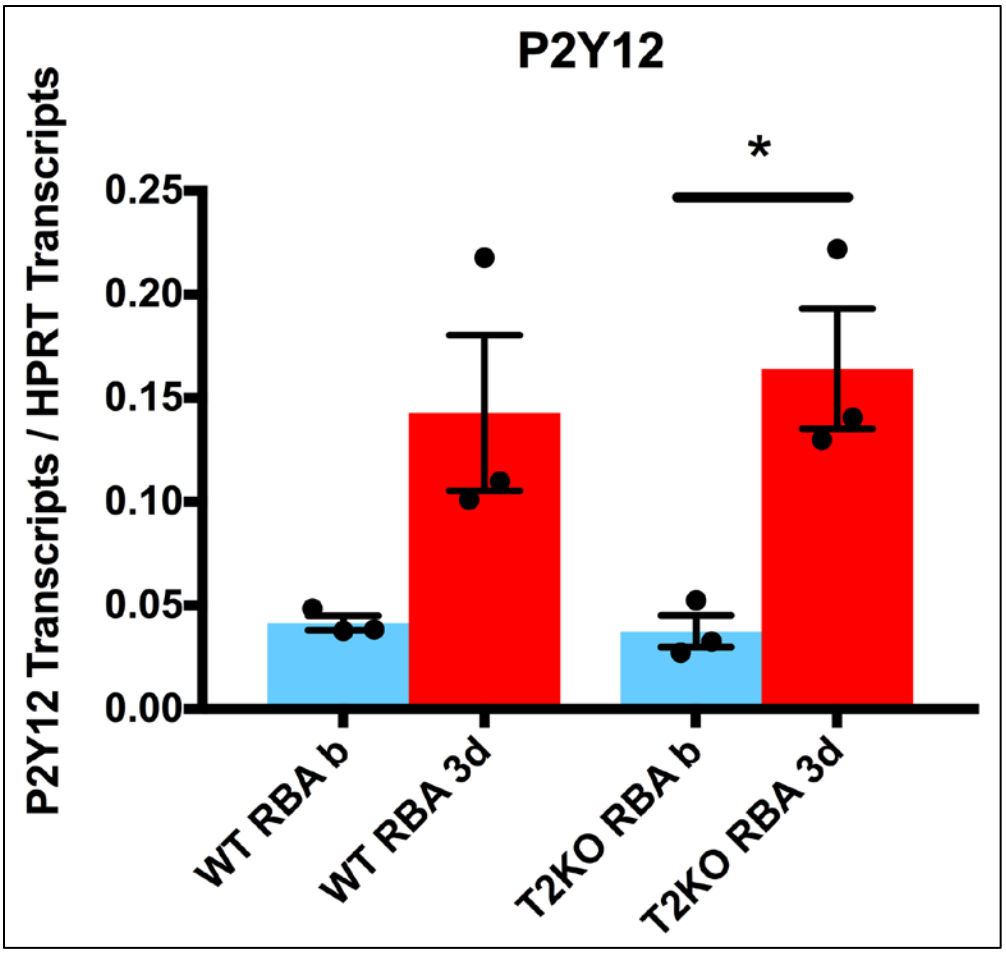


Figure 3: Significant increase in P2Y12 levels in 3 day post TBI mice, when compared to baseline (no TBI) mice for TREM2KO genotype. There is a 4.38-fold increase in P2Y12 transcripts in the TREM2KO 3 day post TBI samples, when compared to the TREM2KO baseline (no TBI) samples. Furthermore, the difference is statistically significant, as denoted by the asterisk. Additionally, there is a 3.44-fold increase in P2Y12 transcripts in the WT 3 day post TBI samples, when compared to the WT baseline samples. Only RBA values are shown because the right hemisphere received the impact and is more representative of reaction to TBI than the left hemisphere.

3. We see a significant increase in TNFa in the impacted hemisphere (RBA) compared to the contralateral hemisphere (LBA) of 3 day post TBI WT mice.

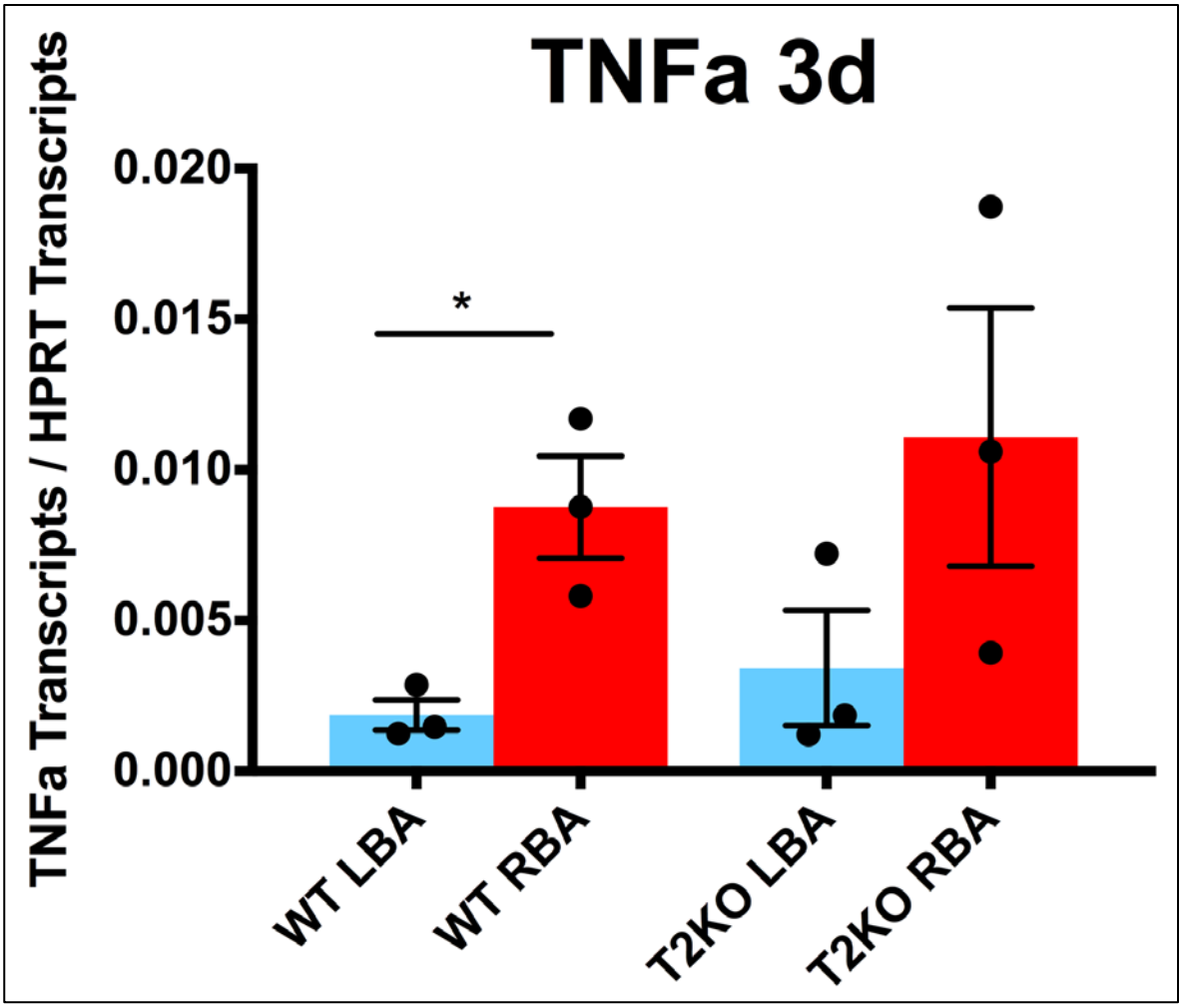
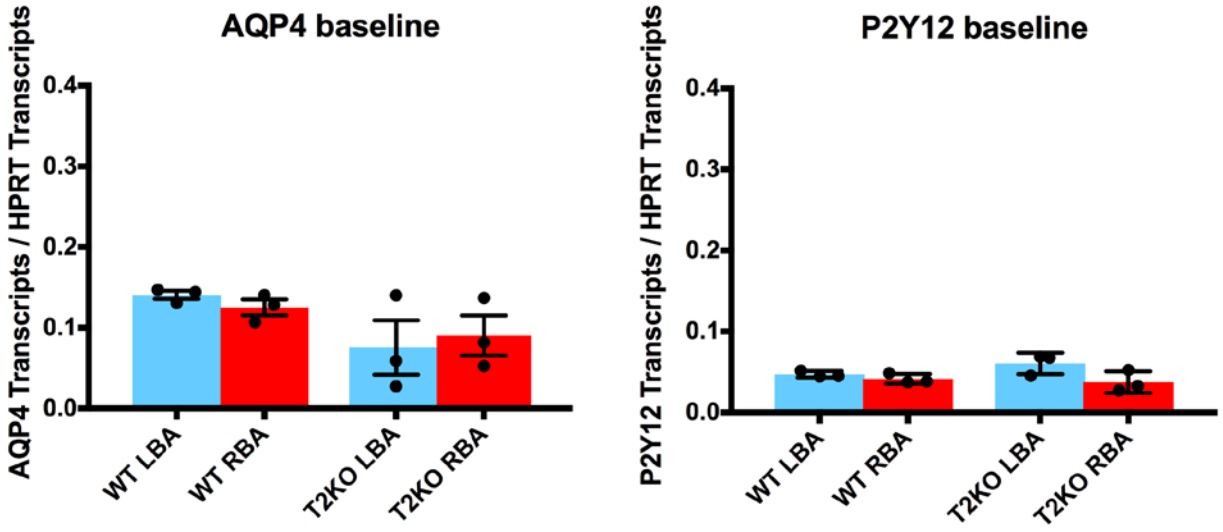


Figure 4: Significantly more TNFa expression in the impacted hemisphere for WT mice. There is a statistically significant difference in TNFa mRNA transcript expression in the impacted hemisphere (RBA), when compared to the contralateral hemisphere (LBA), for wildtype mice. This is not true for TREM2KO mice. However, TREM2KO mice do show a trend towards a similar pattern in the difference of TNFa mRNA transcript expression.

4. As we expect, there is no significant difference in expression levels of all our genes of interest between the left and right hemispheres of our baseline samples. Our baseline samples never received a CCI for TBI, and should show no difference between the left and right hemispheres.



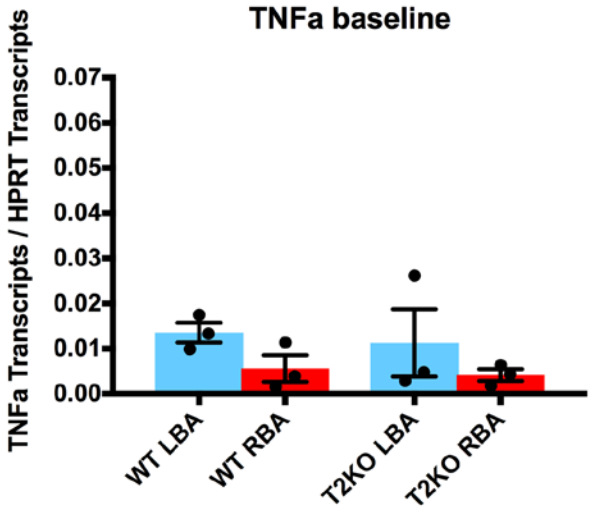


Figure 5: The left and right hemispheres show no significant differences in baseline samples. This result is as expected, because there was no impact to either hemisphere in our baseline samples. We completed this data to show that our model of TBI is accurate.

Conclusion

From this data, we can tell that the brain is still experiencing a lot of damage at the three day post TBI time point. We see increases in expression of P2Y12 in 3 day post TBI WT and 3 day post TBI TREM2KO mice, compared to baseline levels. The presence of above normal levels of P2Y12 is a marker of cell damage near the impact. We also see that TNFa, a pro-inflammatory marker is present in much higher amounts on the impacted hemisphere, compared to the contralateral hemisphere at the three day post TBI time point. These findings have implications for treatment and recovery from traumatic brain injury. Even if the patient feels better three days after the incident, it would be unwise to resume normal or excessive physical activity because the brain is still in the process of coping with the injury it sustained.