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PRINCIPAL INVESTIGATOR: Martin J. Mangino

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Martin J. Mangino, PhD

E-Mail: martin.mangino@vcuhealth.org

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University
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14. ABSTRACT Traumatic brain injury (TBI) caused by blast or projectile injury results in brain swelling from vascular edema and later from intracellular metabolic cell swelling. This causes compression of the microcirculation and lowers tissue perfusion, oxygenation, and more cellular ischemia, which causes more metabolic swelling and microvascular compression in a self-amplifying cycle. We have developed new cell impermeant-based solutions to resuscitate shock patients by reversing this cycle and hypothesized it would work in TBI. Studies in rodents with contusion-induced TBI resulted in significant cell swelling, which was reversed by the use of polyethylene glycol (PEG) 20k based impermeant solutions given immediately after TBI. This was further associated with reductions in neuro-inflammation in the tissue as well as clear benefits to the longitudinal learning and cognitive behavior in the rats after recovery. Specifically, rodents receiving impermeant-based low volume resuscitation containing PEG-20k performed significantly better in the Morris Water Maze test that measures learning compared to controls receiving the same volume of carrier solution.

15. SUBJECT TERMS
Traumatic Brain Injury (TBI), tissue swelling, PEG Polymers, Impermeants, neurotrauma, cognition, rodents

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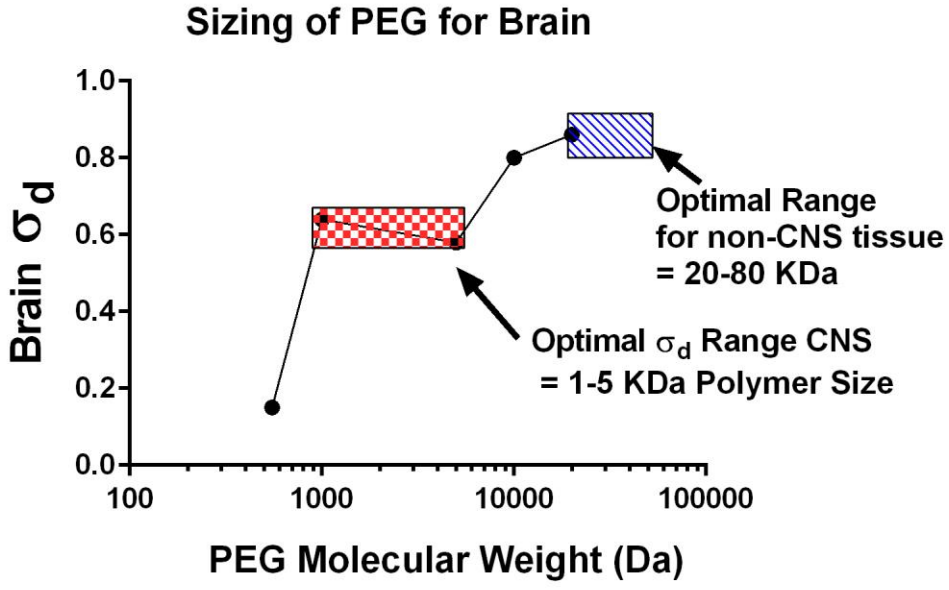
TABLE OF CONTENTS

1. I**ntroduction:** Traumatic brain injury (TBI), the "signature" wound in the Iraq war, by itself or associated with complex trauma is a serious problem for warfighters injured in far forward areas. Progressive brain swelling secondary to closed trauma aggravate the initial injury as does the limitations in transport times to hospitals. Stabilization of traumatized tissues in the brain and in other organ systems is necessary to expand the safe pre-hospital and transport times required to improve outcomes. A new low volume resuscitation (LVR) platform using cell impermeant polymers protects systemic tissues after trauma and ischemia by reversing and preventing lethal cell and tissue metabolic swelling. This is done by establishing multiple osmotic gradients in the microcirculation to drive water flow directly out of cells and by decompressing the microcirculation to allow more efficient oxygen transfer (prevent no-reflow). Since similar metabolic swelling mechanisms occur in TBI, it is reasonable to suggest that this platform of IV solutions is effective for brain swelling and for secondary improvements in microcirculatory perfusion of the brain to improve outcomes.

2. **Keywords**: Traumatic Brain Injury (TBI), tissue swelling, PEG Polymers, Impermeants, neurotrauma, cognition, rodents

3. **Accomplishments:**

PEG Polymer Sizing: (**Project I- Experiment 1, Task 1**). We have extensive experience using polymers of polyethylene glycol in peripheral vascular beds to prevent and reverse metabolic swelling that occurs after trauma. The metabolic swelling serves to reduce local capillary perfusion in the injured tissues by compressing the vessels and limiting oxygen delivery at a time when the tissue is already ischemic. We have determined that the polymer size determines the degree of partitioning of the molecule in the interstitial space (outside the capillary space) versus the amount of the material that partitions in the capillary space. When that ratio is about 0.5 (1 molecule outside for every 2 molecules inside), then water transfer is optimal. The PEG polymer size used systemically for shock has an optimal molecular weight of 20,000 (PEG-20k). However, capillary beds in the central nervous system, including the brain, have "tighter" capillary permeabilities and partitioning coefficients for these molecules. We hypothesized that the size of the PEG molecule that would give similar partitioning ratios as PEG-20k in the peripheral circulation would be much smaller than 20k. Therefore, we determined the proper size of polymer to use in TBI, based on our previous work in the microcirculation in shock. We determined the osmotic reflection coefficients for various sizes of FITC-labeled PEG polymers. This essentially provides us with these partitioning ratios. We compared the amount of FITC-labeled polymer in the blood simultaneously to the amount that was found in the cerebral spinal fluid obtained from the rat 4th ventricle space. When we plot the size of the labeled PEG polymer infused in rats versus (1- the ratios in the blood /CSF), we obtain the osmotic reflection coefficient (σ_d) for that polymer size. **Figure 1** shows that relationship in the brain.



Therefore, from these studies, we believe that the optimal size of PEG polymer that may have optimal water transfer properties may be much shorter than in the peripheral circulation. We will focus on polymers

between 1-5 KDa for brain specific effects and also add this smaller size to the PEG-20k size that is known to be optimal for shock in the peripheral microcirculation. The combination of both sizes will be tested in the polytrauma model of hemorrhagic shock and TBI.

TBI Model: (**Project II -Experiment 1, Tasks 1 and 2**). Addition to sizing the polymer to the brain microcirculation, we have started using the cortical impact model to prepare for testing in TBI rats. Crystal violet staining of the rodent brain two weeks after TBI show classic lesions and neuronal structure in the global images (Figure 2). Our goals will be to characterize TBI on histological evaluations, on immunohistochemical characteristics, and on behavioral, motor, and pain sensitivity characteristics.

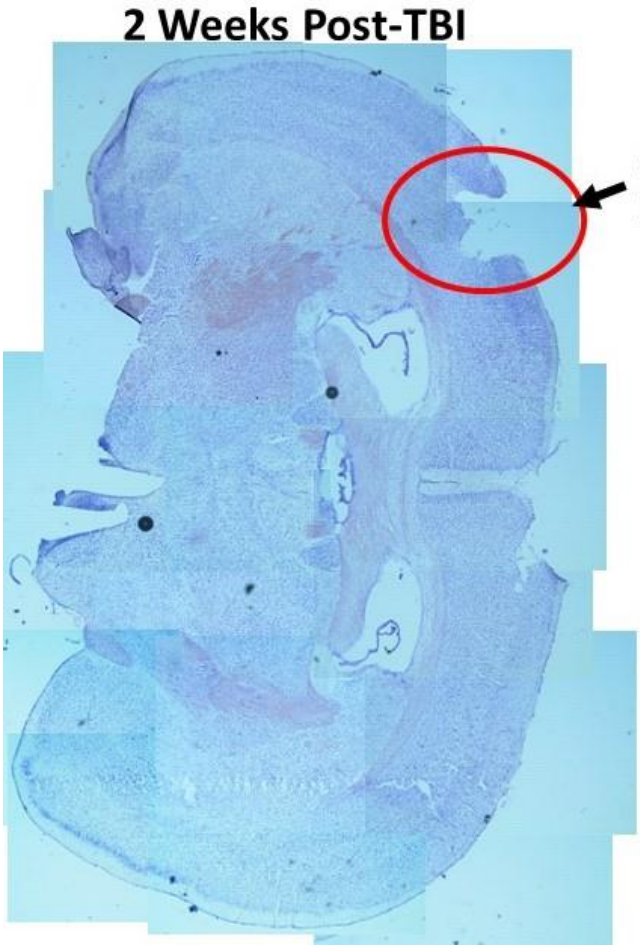


Figure 2

Image shows coronal slice of a rodent brain (30um thick, frozen cryostat section) that underwent TBI two weeks prior to brain tissue collection. Section was stained with cresyl violet to optimize staining protocol and identify injury site (circled).

We used the cortical impact model for testing TBI in rats. Our goals will be to characterize TBI on histological evaluations, on immunohistochemical characteristics, and on behavioral, motor, and pain sensitivity characteristics. But we first need to characterize the biophysical swelling behavior in our model to establish the efficacy of later PEG polymer testing where efficacy first is described by tissue swelling outcomes. We characterized swelling in control-injured animals, which serve as the baseline for polymer tests later. After a cortical lesion caused by the impactor tip on the brain cortex traveling at 5 m/s and penetrating the cortex at a depth of 2 mm is created, the skull and scalp are repaired and the animals allowed to recover from anesthesia with proper analgesic pain control. After 24-72 hours from impact injury, the cortical tissue around the injury site is recovered along with the same hippocampal tissue lying directly under the cortical impact area, and a section of cortex on the contralateral side of the brain are all recovered. The contralateral sample of the cortex is recovered as a paired control sample. Additionally, some rats are subjected to a sham operation where the same surgery occurs as in the TBI rats but no impactor TBI is done. The cortex is removed from these rats and serves as a surgical control. All removed tissue samples are blotted dry on filter paper to remove surface fluids and the wet weights are recorded. After drying in an over (70C) for 36 hours,

the dehydrated tissues are weighed again and the difference is attributable to tissue water. The water weight is divided by the amount of dry tissue weight and the values expressed as TTW. The results of 4-8 experiments in each group are shown in Figure 3 below.

These data clearly show that cortical tissues accumulate a considerable amount of metabolic water after 72 hrs from TBI. This is the rationale for this project. Therefore, we are confident that we now have a water and pressure effect after injury that we can correct with PEG polymer therapy at resuscitation in the field. The fact that hippocampal tissues are not affected is not surprising given the local nature of the injury. The swelling effect between the contralateral control and sham control describes the significant amount of swelling injury that can occur just from the surgical trephination per se, without TBI.

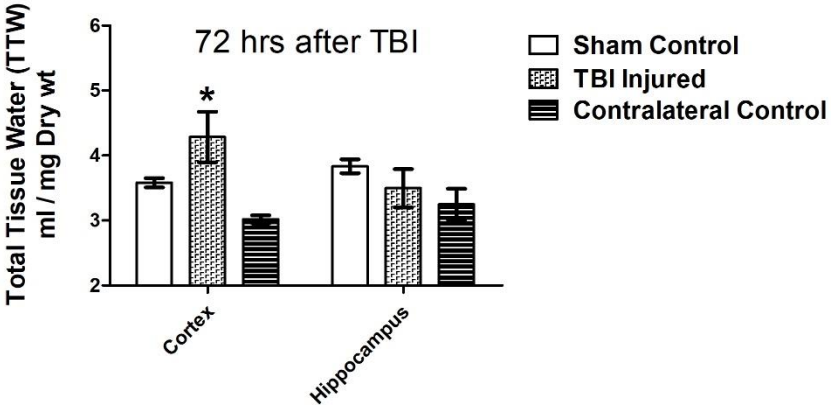


Figure 3. Total tissue water after TBI in cortex and hippocampal regions 72h after **injury**

We next explored how swelling occurs earlier after TBI, which may be most important and clinically relevant because it is at these early times when field resuscitation could have the biggest impact on swelling-induced injury later. So TTW was measured in brain tissue 24 hrs after TBI and the effects of PEG-20k and PEG-5k were assessed.

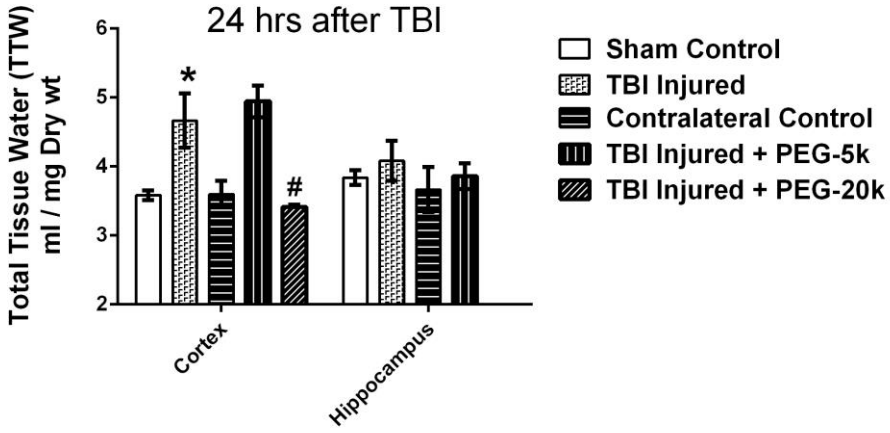


Figure 4. Total tissue water (metabolic cell and tissue swelling) 24 hours after TBI in cortex and hippocampal regions of rat brains. In some studies, a PEG-5k or PEG-20k low volume resuscitation solution was administered after TBI. * P<0.05 relative to Sham and Contralateral, # P<0.05 relative to TBI Injured and TBI Injured + PEG-5k, n=6-8

These data clearly show that cortical tissues have accumulated a considerable amount of metabolic water 24 hrs after TBI. The swelling injury is localized to the surrounding cortical areas and not transmitted to the underlying hippocampus.

Finally, the data clearly demonstrate that administration of a low volume resuscitation solution containing just a 5,000 dalton PEG polymer does not change or reverse the metabolic cell swelling in this model but the 20k polymer does, resulting in a complete normalization of cell water. This result is not particularly shocking because we believe the 20k polymer probably will do most of the work since

the longer polymer has a greater water pull than the smaller 5K polymer, even though the 5k molecule has an optimized osmotic reflection coefficient, which will set up a more effective osmotic gradient. We believe that the combination of 5k with 20k may be most effective and that the effects of polymer treatment will have greatest efficacy in the early phase of injury and with poly-trauma model.

Polytrauma model development- TBI plus Hemorrhagic Shock: (**Project II – Experiment 2, Task 4**). A major goal of this project is to determine the role of PEG polymer on traumatic brain injury outcomes in both TBI alone and TBI with co-existing trauma and shock because that is likely the most common clinical manifestation of TBI in military field medicine and because the two injuries will necessarily potentiate one another. To that end, we have developed a polytrauma shock protocol using our standard rat shock protocol with modifications to accommodate a neurotrauma insult as well. The requirements of this model is that it produce the greatest **survivable** traumatic and metabolic injury as possible. Survivability is key because the TBI injury component requires chronic development and monitoring. Preliminary studies show progress on a new model modified from the original lethal model by adjusting downward the amount of oxygen debt delivered during hemorrhage. We moved the lactate value that would trigger resuscitation from 10 mM to 7 mM. This requires an average blood loss of about 30% of the estimated total blood volume of the rat. The model nicely produces a severe metabolic and cardiovascular derangement that is 100% survivable on its own and after the traumatic brain injury. **Figure 5** below shows the arterial pressure response in the two models after the saline low volume resuscitation is administered to the rats but before TBI is induced.

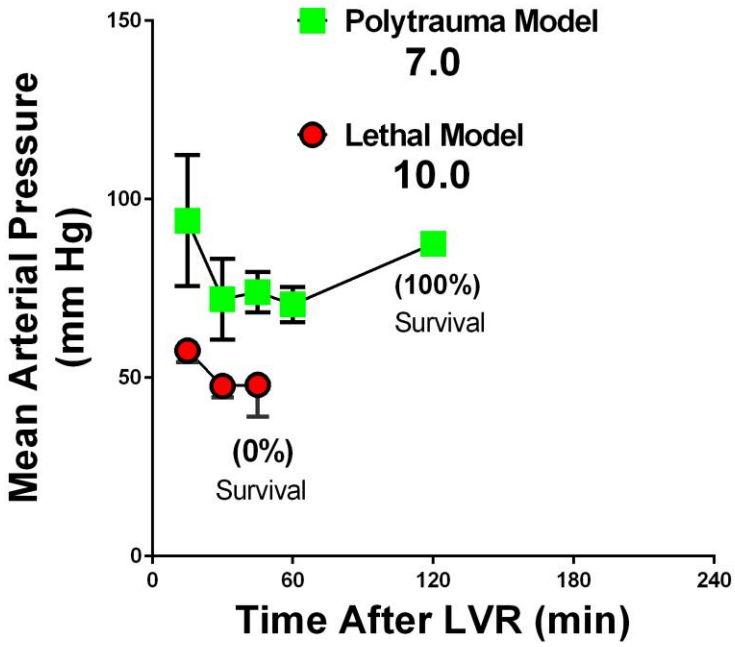


Figure 5: Two shock models developed for studies using newly developed PEG polymer based crystalloid LVR solutions. The polytrauma model will be used in this study to assess the effects of both trauma and traumatic brain injury and how early field resuscitation with PEG polymers can alter these outcomes.

In the first year we characterized the theoretical optimum polymer size for achieving the best result in moving water out of the metabolically swollen brain after injury. We documented that the brain cell and tissue swelling in the cortex induced by TBI could be reversed by PEG polymers given immediately after TBI. We discovered that, despite having a less than optimal partitioning ability, the larger 20k polymer size does much better than the smaller 5k polymer size. We also recalibrated the shock component of the polytrauma model that will allow us to do testing on, not only traumatic brain injury per se, but also TBI in an animal with co-existing cardiovascular and metabolic trauma (shock) in a clinically relevant polytrauma model.

Water Removal from the Brain: (**Project II – Experiment 1, Tasks 1 and 2**). We have determined that PEG-20k and smaller PEG polymers (2k and 5k) used with PEG-20k are able to effectively move water out of areas of the cerebral cortex and underlying hypocampus following TBI. However, these assays were invasive and required death of the rats and single instance sampling to measure total tissue water content. Therefore, we have developed non-invasive high-resolution MRI models to detect water transfer and velocity mapping of water in brain in rodents before and after TBI. In early studies, young adult rats were put under anesthesia using isofluorane. Baseline MRI imaging was obtained to demonstrate normal anatomy of animal and to verify desired structures (including the hippocampus) could be identified. Rats then underwent cortical contusion impact at 5 m/s with a depth of 1-2 mm. A 10% blood volume of lactated ringers was administered 30 minutes after injury. MRI images were obtained, which demonstrate increased T2 weighted signaling beneath the area of the craniotomy, indicative of cerebral edema. 30 minutes after original lactated Ringers infusion, a 10% blood volume of PEG-20k was administered and the animal was re-scanned. A representative example of one experiment is shown in Figure 6.

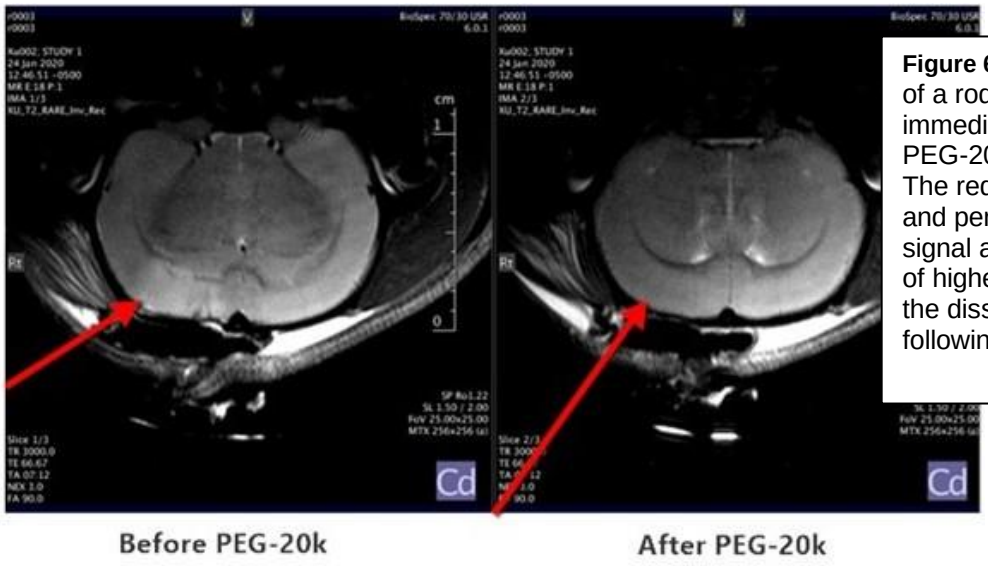


Figure 6. MRI T2 weighted imaging of a rodent with TBI before and immediately after an infusion of PEG-20k resuscitation solution. The red arrow shows the impact and peri-lesional areas. White signal areas indicate tissue zones of higher water concentration. Note the dissipation of the water signal following PEG infusions

Our next pilot experiments will use velocity mapping modes of MRI to determine,.directional movement of water in the tissues and we will examine the effects of TBI and PEG-20k treatment on longitudinal changes in water movement as indexed by MRI over several days. These changes will also be measured in rats with co-occurring TBI and polytrauma induced by hemorrhagic shock induced before immediately TBI. A large amount of time was expended in determining the protocol for MRI imaging and the sequencing of events relative to imaging and induction of injury in the models. In this year, we have accumulated several MRI scans on several rats in each group. The MRI data are being analyzed and processed for quantitative analysis now and will be available in the next quarter. Briefly, the results show that PEG-20k is highly effective at transferring isotonic water out of the TBI injured brain tissues.

Neurobehavioral Testing: (**Project II – Experiment 3, Task 2**). Other studies that were started included establishing the Morris Water Maze (MWM) testing assay. Briefly, the MWM test is used to determine a primary outcome of traumatic brain injury in the rodent model so we can assess if polymer infusions influence the outcomes. The Morris water maze task is a well-validated test for

spatial learning, memory, and behavior. The testing area is a round pool filled with water where a hidden platform is submerged just below the water surface and hidden by a dark pigment in the water. The rat is introduced into the water tank, swims, and learns to escape from the water by locating the hidden platform, mainly by the use of visual cues located in the room with symbols on the walls that serve as special reference points. Alternatively, the platform can be placed in another quadrant, or removed during another phase of the experiment. This way, memory retention and extinction can be investigated. Typically, the forward acquisition time is the time that is required for the rat to find the platform to escape the water after being released in to a quadrant of the pool across from the quadrant where the platform is located. This time is determined on the first day in the pool and then on subsequent days for about a week. As time passes, the rats learn through visual and special cues where the platform is located so they find it faster each day as they learn. These learning kinetic curves can be compared between various groups of rats including negative controls (rats without TBI), positive controls (rats with TBI, usually measured 5 days after injury and surgical recovery), and experimental groups that include rats with TBI that have undergone treatment (PEG-20k). In establishing the equipment and the behavioral model, we first studied naïve rats without any injury to generate forward acquisition curves. Figure 7 demonstrates two forward acquisition curves from 2 independent naïve rats measured over 5 days.

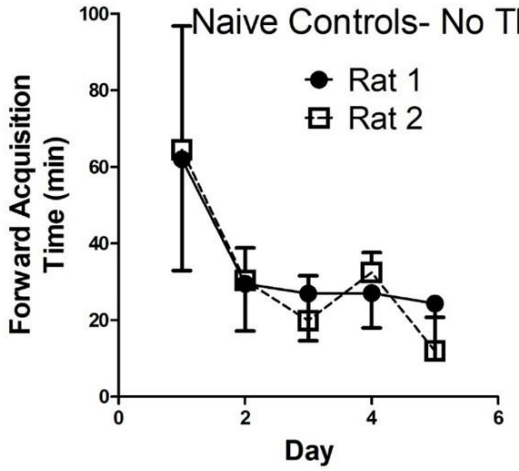


Figure 7. Morris Water Maze performance curves in two normal rats without TBI that were used to validate the assay and to establish normal learning acquisition curves in rats without TBI. Other groups of rats with TBI will be compared to these curves to determine the TBI learning defect and to establish the positive control to compare PEG-20k interventions. These data from each rat represent an average and variance from 4 repetitive tests on each day. Note the rapid decrease in acquisition time each day that represents the normal fast rate of learning and special memory in uninjured rats.

Cognitive testing in TBI: (**Project II – Experiment 3, Task 1 and 2**). Once we confirmed the Morris Water Maze (MWM) procedure for rodents was working well, we began testing rats with TBI that received either the Lactated Ringer's solution control (LR) or PEG-20k IV solution. Both were administered after TBI at a volume of 6.8 ml/kg. Preliminary results in 6 rats per group are shown in Figure 8. For the first 10 days, the rats were allowed to swim to the same platform location. On the 10th day and thereafter, the platform was moved to a different location and the rats needed to find it again and extinguish the previous memories of the location and cues associated with the first platform. The results indicate that at all time points, the rats treated with PEG-20k learned quicker than the rats with TBI treated with LR solution as a volume control. At some days after TBI, the time to find the platform was cut in half by PEG-20k

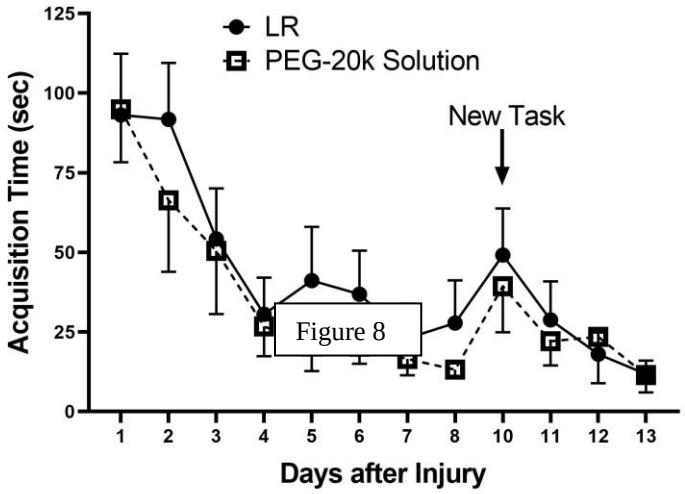


Figure 8

treatment. They also learned quicker after the platform was relocated. Because the statistical power for this test was calculated to be about 12 or more rats per group, we did not perform a statistical analysis. We have focused on repeating the experiments to determine real differences.

During the second year, we have made progress in testing the PEG-20k containing resuscitation solutioin in a key test for cognitive and learning behavior in rodents suffering from TBI. The Morris Water Maze (MWM) test works well in our model and demonstrated initial efficacy of our first group of rodents treated with PEG-20k. At every day after TBI, rodents learned faster in the bMWM test compared to their control group treated only with LR solutiuon (the vehicle for PEG-20k). In some days, the differences in learning speed between the two groups was two fold. Finally, we have also produced a large volume of MRI scans in rodents with TBI. Although the scans are currently being analyzed and quantitated for water flux and water content in the brain injury sites, we can often tell by the naked eye that PEG-20k is dramatically reducing the water signal in the brain injury site in rats after TBI. Our detailed analysis will be available soon.

TBI-Induced Inflammation: (**Project II – Experiment 3, Task 4**).

Rats that were randomized to resuscitation with either LR vehicle of PEG-20k IV solution both given at 6.8 ml/kg, IV after TBI. Brains were recovered from the rats 72 hours after TBI for analysis of GFAP expression in the tissues. The following areas of brain were removed and embedded in paraffin blocks;

1. Medial Superficial Perilesional area
2. Medial Deep Perilesional Area
3. Medial Lesion
4. Middle Lesion
5. Lateral Lesion
6. Lateral superficial Perilesional area

Paraffin embedded sections were de-paraffined, de-cloaked for antigen retrieval, and incubated with a fluorescent anti rat GLAP antibody. The signals in the tissues were imaged and captured with a fluorescent microscope and the positive area analyzed with ImageJ. The positive areas of the brain expressing GLAP were normalized to percent area. The results of the GFAP expression are shown in Figure 9.

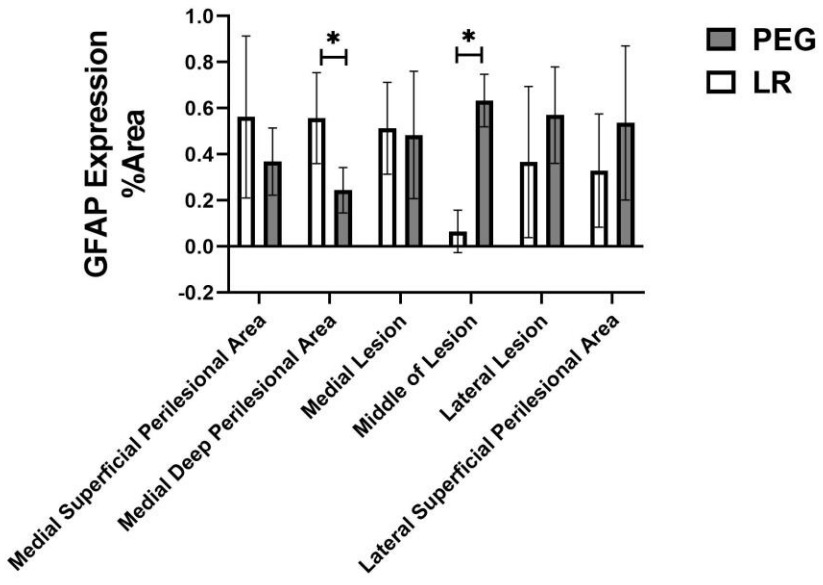


Figure 9. Expression of the astrocyte protein GFAP in areas of rat brains that had experienced TBI 72 hours earlier. GFAP expression is an index of the inflammatory state of the astrocytes in the brain regions around the lesion created by the TBI impactor. The deep medial perivascular region shows suppressed inflammation in the PEG group while the GFAP is 10 fold higher in the PEG group in the middle of the lesion. Other areas are not different. *P<0.05 between groups, n=6

These data indicate that there is less inflammation associated with astrocyte activation in the regions of the brain that are both medial and deep to the injured area. If this involves the deeper hippocampus structure, then this may explain why we might see outcomes related to spatial learning in this model, which is what we are testing with the Morris Water Maze test. These data also show a 10 fold less inflammation signal in the area in the middle of the lesion. While we could interpret this to mean that the PEG treatment caused more astrocyte inflammation, we could also hypothesize that the differences between the LR control and the PEG-20k treated signals in this area are due to a much lower signal in the LR group. While LR would not realistically be associated with an anti-inflammatory event after TBI, it may represent loss of protected effects of the PEG. We therefore think that it is more reasonable to suggest that PEG protected the neurons in this area of the lesion more so than the control and that the loss of signal in the LR group is attributable to a greater loss of neuronal mass, including astrocytes that express GFAP. Addition al experimnents need to be designed to test this hypothesis further.

Spatial learning and cognitive function: (**Project II – Experiment 3, Task 1, 2, and 3**)
Rats receiving low volume resuscitation with either LR solution (control) or PEG-20k IV solution at the same volume after TBI were used in these studies. The purpose was to determine if rats getting PEG-20k have improved spatial learning and cognitive function. The mechanistic hypothesis is that cell impermeant therapy with PEG-20k non-energetically moves metabolic water (accumulated during the traumatic injury) from neurons into capillaries where it is swept away by convective solvent transfer (blood flow). This reduced tissue swelling decompresses the local capillaries and more efficient oxygen exchange occurs after injury, leading to less downstream neuronal injury and faster recovery. To test this, we used the MWM test. One day following recovery from TBI, rats were placed into a quadrant of the MWM test swimming pool (6 ft diameter). A platform for the rats to escape the pool is located in the NW quadrant just below the water surface so they can climb out of the water. Colored paint (black) is added to the water to hide the visual presence of the platform. Large brightly colored pictorial symbols are place along a curtain that circumscribes the swimming pool to allow the rats to see constant visual cues to help them locate their position while in the pool. Rats are first trained to find and stay on the platform for 7 days. On the 8th day, each rat is "probed" for their visual and cognitive ability using various outcomes to judge their ability to find the platform. A camera above the tank records their swim path, distance, route, and times. Software later interprets these variables to assess learning function. The results of preliminary studies and analysis of these data for the "probe" part of the testing is shown in **Figure 10.**

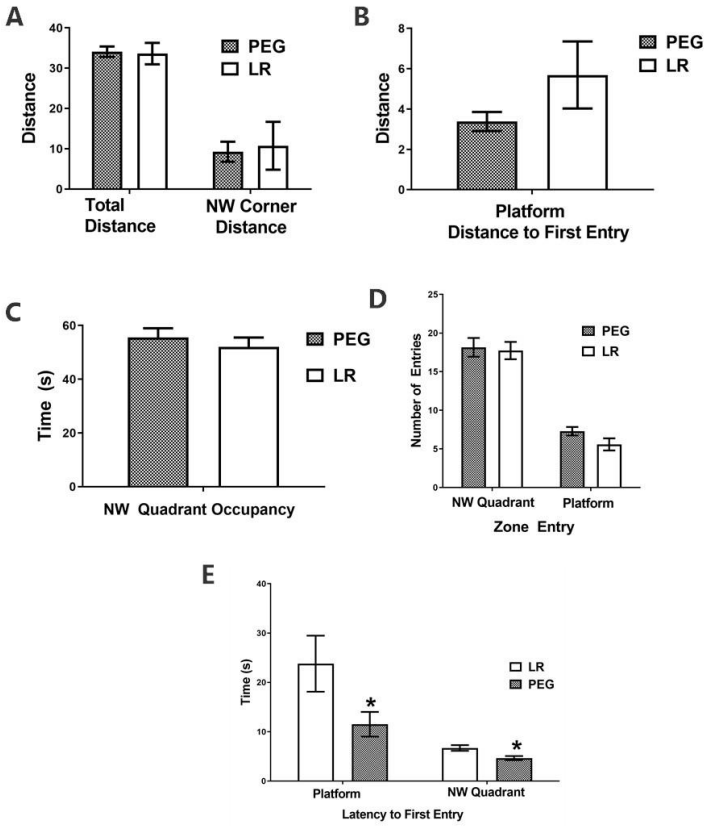


Figure 10. Probe outcomes of the Morris Water maze test in rats with TBI either resuscitated with LR solution (control) or PEG-20k IV solution. Rats were trained for 7 days starting one day after TBI and probed on the 8th day for spatial learning behavior. The escape platform was always located in the NW quadrant of the pool. **A**. Total distance traveled and distance spent in the NW quadrant. **B**. Distance to first entry into the NW quadrant, **C**. Time in the NW quadrant, **D**. Number of times rats entered the NW quadrant of the platform, and **E**. Amount of time it took rats to enter the platform and NW quadrant from the time of swimming. N=10 rats per group, *P<0.05 relative to LT, values are mean ± SD.

Analysis: These data suggest that there are significant spatial learning and cognitive behavior improvements in rats resuscitated with PEG-20k IV solution compared to the vehicle controls after TBI. Specifically, they learned and located the platform twice as fast as the controls. Since this action requires superior visual que processing and learning behavior, we believe that hippocampus and thalamus function is better after TBI. Executive cortical control may also be improved since these all are needed to improve learning behavior. This is consistent with the earlier observations that the deeper perilesional structures are less swollen and inflamed after TBI in the PEG-20k resuscitated rats relative to the LR controls. These deeper layers are consistent with hippocampus areas.

We previously showed MRI evidence of water transfer in rat brains after TBI and how this was affected by treatment with PEG-20k IV solution. In previous studies earlier in the project, we definitively demonstrated metabolic cell and tissue swelling in TBI injured regions of the brain and resolution with PEG-20k IV solution resuscitation. However, using MRI we have different data.

Adult anesthetized Sprague Dawley rats were first scanned in the MRI to establish each rat's baseline T1 weighted Apparent Diffusion Coefficient (ADC) values. The tissue ADC represents rate of movement of water across the tissue and can detect metabolic disturbances in water management by these cells. The ADC T1-weighted images show low ADC values coded as darker than higher ADC values. The slower water movement is indicative of more water in these tissues, because it is not moving. A typical scan of a series of 3 rats in the control group (**Figure 11**) shows the metabolic swelling defect as dark regions in and around the impact site at the top right of the brains.

Figure 11

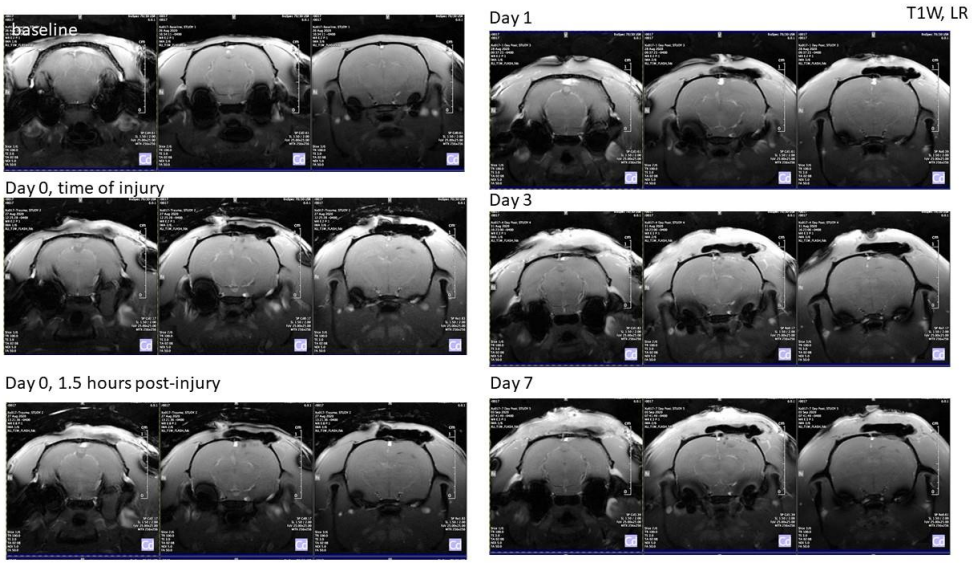


Figure 11. Three independent rat studies showing the ADC values (coded in shades of grey) in coronal sections of the MR imager. Each panel shows 3 rat brain images at the indicated time after TBI. All of these animals served as positive controls and were treated only with a resuscitation IV consisting of lactated ringers solution given at 6.8 ml/kg body weight, IV immediately after TBI. The upper left cuts show the baseline images for three rats. Middle left show the same rats immediately following the impact injury. Lower left show scans after 1.5 hours. Upper right shows scans after 1 day, middle right after 3 days, and lower right after 7 days. The dark areas seen on the top of the images after impact are the damaged tissues, representing lower ADC values relative to the surrounding tissues.

A quantitative representation of these data from two groups receiving either the LR vehicle as a control or the PEG-20k IV solution test solution are shown in **Figure 12**, from 5 rats in each group. The upper left panel shows ADC values from rat brain slices obtained through the cortex in the hemisphere that was NOT injured. The upper right panel shows the same from the injured hemisphere. The bottom right shows ADC values from slices through the injured thalamus regions and the lower left panel is the same but from the non-injured thalamus regions. Each time point shows the corresponding ADC values from the baseline value, the value from rats treated with the LR vehicle, and the last bar from rats treated with the PEG-20k IV solution.

Figure 12

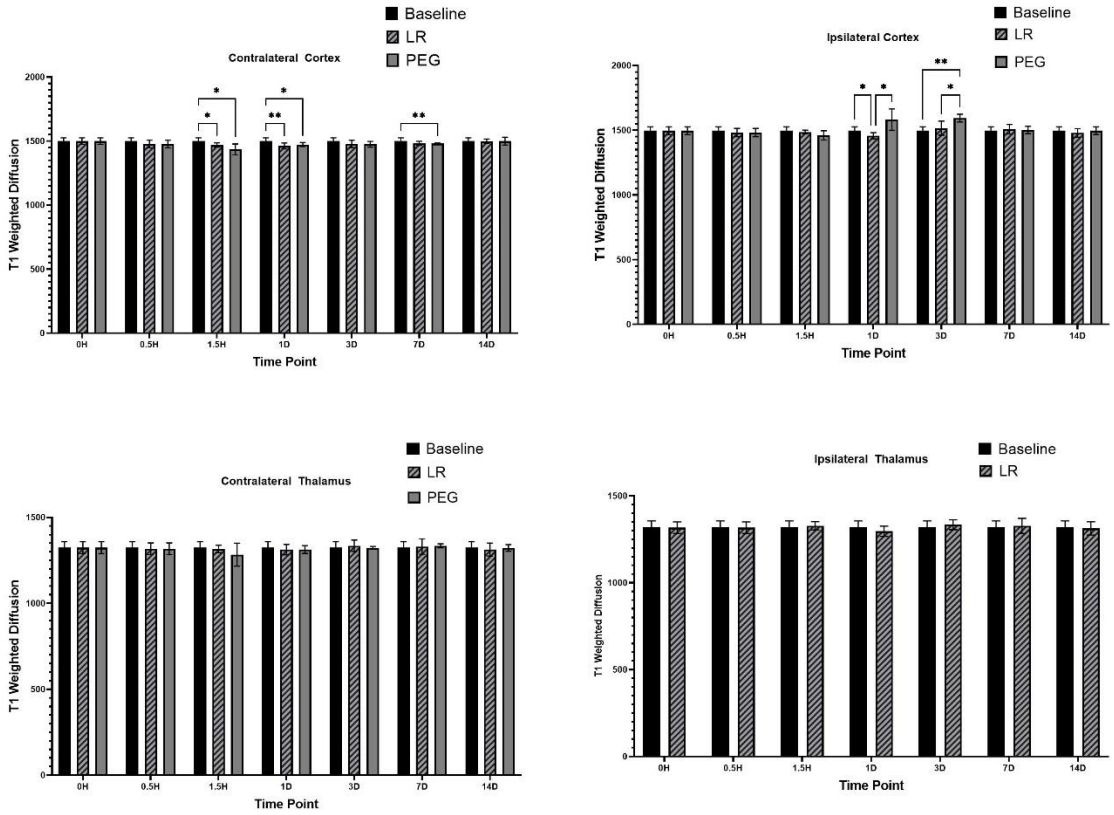


Figure 12.Apparent Diffusion Coefficients (ADC) from MRI scans of rat brains after TBI injury over time from baseline, control (LR treated), and PEG-20k IV solution treated rats. The values are averages from the group of 5 rats each. Error bars are standard deviation.

Analysis: These experiments show that TBI in rats performed under the conditions in our lab produce regional areas of the cortex in both the ipsilateral and contralateral (to a lesser degree) that have significantly lower ADC values, relative to surrounding tissue and to the same areas at the baseline before injury. This occurs over a 1-week period and is seen as early as immediately after impact injury (about 30 minutes for the scan time after injury). This only is seen in the underlying cortical areas of the impact and not the thalamus regions of the brain. Finally, these lower ADC regions are representative of areas with more cell swelling and less diffusion of water in and out of the tissues. These data, in contract to our previous data that estimated total tissue water in these regions by a physical biopsy method, show no difference between the vehicle and the PEG-20k treated groups, but do nicely show the positive control of injury, relative to the baseline values.

4. **Impact**

Studies conducted in this period tested the overall hypothesis that TBI causes metabolic cell and tissue swelling by disruptions in brain bioenergetics, which cause compression of the brain microcirculation and further reduce perfusion causing more metabolic swelling in a self-amplifying cycle. Since we have developed powerful molecular research tools to test this by osmotically reversing metabolic tissue swelling (which isn't the same as tissue edema) we also are able to measure it's therapeutic potential in treating TBI immediately after injury. This is useful because stopping a positive feedback cycle that causes loss of tissue perfusion after injury will help maintain or restore brain function later by maintaining the delivery of oxygen to active neurons. Furthermore, since this resuscitation strategy is also useful in treating traumatic hemorrhagic shock, it ncan be useful in Polytrauma patients that often co-express both systemic shock and TBI. Finally, these resuscitation solutions containing the primary osmotically active polymers are stable in the field and can be used immediately at the time and point of injury in the pre-hospital setting. This early treatment and early preservation of brain oxygen transfer is key to short-circuiting later developments of classic constellations of symptoms associated with TNI in patients and injured military personnel.

5. **Changes/Problems**

The biggest problem encountered in this project period was the lack of productivity that resulted from the COVID-19 crisis of 2020. The lab lost productivity to direct shut down of the university and lab for 6 months in early 2020 as well as follow on disruptions throughout the next year that occurred from loss of university services for animal ordering and care because of COVID quarantine policies, which resulted in severe staff shortages and supply chain delays in key reagents and supplies for the study. Consequently, the 3-year project only represents about 2 years of productive work. Therefore, the TBI combined with circulatory shock studies (**Project II – Experiment 4**) were never completed after the model was initially established (**Project II – Experiment 2**).

6. **Products:**

The biggest product is impending FDA issuance of an IND to begin trials of the research tools used in this study for TBI in hemorrhagic shock. The company commercializing the solution is planning to seek a labile expansion to patients with TBI alone or as part of Polytrauma by conducting clinical trials in a Phase IIa pilot study. This will be in approximately 2-3 years.

7. **Participants & Other Collaborating Organizations:** The following personnel have participated at some time during this project.

Name: Martin Mangino, PhD
Project Role: PI
Researcher Identifier:
Nearest person month worked: 3.6
Contribution to Project: Dr. Mangino is supervising all of the studies, analyzing data, assists with animal surgeries, and is preparing statistical analysis, reports, and manuscripts.

Name: Kirsty Dixon, PhD
Project Role: Co-I
Researcher Identifier:
Nearest person month worked: 3.6
Contribution to Project: Dr. Dixon is supervising the studies and evaluating all of the histological assessments and tissue molecular work.

Name: Nancy Lee
Project Role: Tech.
Researcher Identifier:
Nearest person month worked: 6
Contribution to Project: Nancy helps with the injury surgery and animal care for the project.

Name: Caitlin Archambault, BA, LVT
Project Role: Lab Manager
Researcher Identifier:
Nearest person month worked: 3
Contribution to Project: Caitlin manages the daily routine of the lab and orders supplies, assigns tasks, organizes data and documents, maintains the lab compliance for the project, and helps with veterinary issues for the animals.

Name: Anna Xu, MD
Project Role: PGY2 General Surgery Resident (Research Fellow)
Researcher Identifier:
Nearest person month worked: 6
Contribution to Project: Dr Xu is new to the project since July 2019. She rotated into the lab from her general surgery residency where she worked on this project and others for the last 2 years. She has prior training in neurotrauma and research in this area. She lead much of the work in the lab on this project under the direction of Dr. Mangino and Dr. Dixon.

Name: Jerry Maitland, BS
Project Role: Lab Technician
Researcher Identifier:
Nearest person month worked: 12
Contribution to Project: Jerry was a new research technician working on this project for a year. He has prior experience in neurotrauma and entered the PhD program in the fall of 2020.

Name: Niama Rihane, PhD
Project Role: Post-Doc
Researcher Identifier:
Nearest person month worked: 6
Contribution to Project: Niama worked on the project for the first year only as a post-doctoral training. She conducted experiments, analyzed data, and wrote reports

Name: Jad Khoraki, MD
Project Role: Post-Doctoral fellow
Researcher Identifier:
Nearest person month worked: 3
Contribution to Project: Jad provided surgical expertise for a year on the project for the Polytrauma experiments

Name: Loren Liebrecht, MD
Project Role: Post-Doctoral Fellow
Researcher Identifier:
Nearest person month worked: 3
Contribution to Project: Loren provided animal handling and data collection and analysis help on the project for the last year.

8. **Special Reporting Requirements:**

9. **Appendices:** None