Published in final edited form as:

Brain Inj. 2016 ; 30(2): 217–224. doi:10.3109/02699052.2015.1090012.

Experimental diffuse brain injury results in regional alteration of gross vascular morphology independent of neuropathology

Jenna M. Ziebell^{1,2,*}, Rachel K. Rowe^{1,2,4}, Jordan L. Harrison^{1,2}, Katharine C. Eakin^{1,2}, Taylor Colburn^{1,2}, F. Anthony Willyerd^{1,2,3}, and Jonathan Lifshitz^{1,2,4,5}

¹BARROW Neurological Institute at Phoenix Children's Hospital, Phoenix, AZ

²Department of Child Health, University of Arizona College of Medicine - Phoenix, Phoenix, AZ

³Critical Care, Phoenix Children's Hospital, Phoenix, AZ

⁴Phoenix VA Healthcare System, Phoenix, AZ

⁵Psychology, Arizona State University, Tempe, AZ

Abstract

Primary objective—A dynamic relationship exists between diffuse traumatic brain injury and changes to the neurovascular unit. The purpose of this study was to evaluate vascular changes during the first week following diffuse TBI. We hypothesized that pathology is associated with modification of the vasculature.

Methods—Male Sprague-Dawley rats underwent either midline fluid percussion injury or sham-injury. Brain tissue was collected 1d, 2d, or 7d post-injury or sham-injury (n=3/time point). Tissue was collected and stained by de Olmos amino-cupric silver technique to visualize neuropathology, or animals were perfused with AltaBlue casting resin before high-resolution vascular imaging. The average volume, surface area, radius, branching, and tortuosity of the vessels were evaluated across three regions of interest.

Results—In M2, average vessel volume ($p<0.01$) and surface area ($p<0.05$) were significantly larger at 1d relative to 2d, 7d and sham. In S1BF and VPM, no significant differences in the average vessel volume or surface area at any of the post-injury time points were observed. No significant changes in average radius, branching, or tortuosity were observed.

Conclusions—Preliminary findings suggest gross morphological changes within the vascular network likely represent an acute response to mechanical forces of injury, rather than delayed or chronic pathological processes.

*Corresponding Author: Jenna M. Ziebell, PhD, Scientist, Translational Neurotrauma Research Program, BARROW Neurological Institute at Phoenix Children's Hospital, 1919 E. Thomas Rd. | Ambulatory Bldg., 3rd Fl. | Phoenix, AZ 85016, p (602) 827-2349 | f (602) 827-2257, Jenna.Ziebell@utas.edu.au.

Declaration of interest:

The authors report no declarations of interest. Research reported in this manuscript was supported, in part, by National Institute of Neurological Disorders and Stroke of the National Institutes of Health under award number NIH R01 NS-065052, and Phoenix Children's Hospital Mission Support Funds.

Keywords

neurovascular unit; traumatic brain injury; neuropathology; vascular casting; midline fluid percussion

1. Introduction

Traumatic brain injury (TBI) is a major cause of death and disability throughout the world ^{1,2}. Despite improvements in critical care, morbidity and mortality remain high. TBI is a heterogeneous disorder with varying symptoms based on mode and severity of injury. It can lead to short and long-term cognitive and behavioural deficits and increases the risk for neurodegenerative disease ³⁻⁶. Injury can be divided into two phases: the primary mechanical damage is irreversible and only amenable to preventative measures to minimize the extent or incidence of injury including the wearing of seat belts and helmets and reducing vehicle speed; the second phase is a multifactorial process initiated at the time of impact and evolving over the subsequent hours to days. Secondary injury leads to physiological, cellular and molecular responses aimed at restoring homeostasis, which if not controlled, will lead to further injury.

The neurovascular unit of the brain is comprised of neurons, astrocytes, oligodendrocytes, microglia, endothelial cells and pericytes ⁷. Disruption to this neurovascular unit can lead to local and global events which affect neurological function and overall outcome ⁷. Processes involved in the secondary phase of injury are under ongoing investigation as targets for novel therapeutic strategies to limit delayed brain damage. These strategies often aim to reduce cell death, inflammation and behavioural morbidities ⁸⁻¹². However, limited literature is available on injury-induced alterations to the vasculature. Pathological changes to the vasculature, such as redirection of blood flow, blood-brain barrier (BBB) permeability, angiogenesis, and oedema may propagate secondary injury cascades and worsen outcome ¹³.

Midline fluid percussion injury (FPI) can produce a concussion-like diffuse brain injury in rats, characterized by brief neurological and systemic physiological alterations without remarkable structural damage ¹⁴. Furthermore, it has been demonstrated that this model is associated with decreases in cerebral blood flow ¹⁵. With decreased blood flow, endothelial cells of the BBB become distressed, leading to disruption of the BBB and the influx of solutes and proteins from the periphery, defined as vasogenic oedema ¹⁶. The period of vasogenic oedema is followed by cytotoxic oedema, where cells such as astrocytes take up excess fluid to restore homeostasis ¹⁶. With the transient opening of the BBB following diffuse brain injury ¹⁷, both vasogenic and cytotoxic oedema can occur ^{18,19}. Oedema has been associated with poor outcome following TBI, predominantly by raising intracranial pressure, impairing cerebral perfusion and oxygenation ¹⁶. These data suggest that vascular remodelling may be a meaningful component of delayed brain injury processes. To this end, a study of lateral FPI, where cavitation and contusion occur, reported a diffuse and heterogeneous change in the microvasculature ²⁰. Decreased capillary density and diameter as well as reduced perfusion of microvessels were reported. Regardless of injury severity, there was a reduction in the density of microvasculature at 1 day post-injury. However, by 2

weeks post-injury density was comparable to sham rats, indicative of recovery. The authors speculated that these changes in vasculature may exacerbate pathology and ultimately outcome.

On the other hand, midline FPI results in rapid secondary axotomy within the cortex and thalamus without cell death^{21–24}. These physical forces which can tear axons may similarly tear blood vessels. Our previous studies using midline FPI have demonstrated temporal and regional pathology as well as circuit dysfunction in discrete regions such as the primary somatosensory barrel field cortex (S1BF) and ventral posteromedial thalamus (VPM)^{25–30}. The pathways between these two regions control whisker somatosensation^{31,32}. Damage to the S1BF and VPM manifests as an increased behavioural sensory sensitivity to manual whisker stimulation^{25,27}. In fact, whisker stimulation in the chronic period following midline FPI produces functional hyperactivation of the S1BF and VPM³³. Midline FPI-induced pathology in the thalamocortical pathway is associated with increased numbers of activated microglia, indicative of local inflammatory cascades, likely contributing to secondary injury processes^{30,34}. Despite these overt changes to cells of the neurovascular unit, no research has investigated the morphological alterations to the vasculature in these regions following midline FPI. Here, we explore whether midline FPI modifies the vasculature in brain regions which are known to harbour pathology associated with behavioural morbidity. We hypothesize that pathology is associated with gross modification to the vasculature.

2. Methods

2.1 Surgical preparation and diffuse brain injury

Adult male Sprague-Dawley rats (350–375 g) were subjected to midline fluid percussion injury (mFPI) consistent with methods described previously^{23,25–27,35,36}. Briefly, rats were anesthetized with 5% isoflurane in 100% O₂ prior to the surgery and maintained at 2% isoflurane via nose cone. Rats were placed in a stereotaxic frame and a midline scalp incision was made to expose the skull. A 4.8-mm circular craniectomy was performed (centered on the sagittal suture midway between bregma and lambda) without disrupting the underlying dura or superior sagittal sinus. An injury hub was fabricated from the female portion of a Luer-Loc needle hub, which was cut, bevelled, and scored to fit within the craniotomy. A skull screw was secured in a 1-mm hand-drilled hole into the right frontal bone. The injury hub was affixed over the craniotomy using cyanoacrylate gel and methyl-methacrylate (Hygenic Corp., Akron, OH) was applied around the injury hub and screw. The incision was sutured at the anterior and posterior edges and topical Lidocaine ointment was applied. Animals were returned to a warmed holding cage and monitored until ambulatory.

For injury induction, animals were re-anesthetized with 5% isoflurane 60–90 min after surgery. The dura was inspected through the injury-hub assembly, which was then filled with physiological saline and attached to the male end of the fluid percussion device (Custom Design and Fabrication, Virginia Commonwealth University, Richmond, VA). As the rat's reflexive responses returned, a moderate injury (1.9–2.0 atm) was administered by releasing the pendulum onto the fluid-filled cylinder. Animals were monitored for the presence of a forearm fencing response as well as the return of the righting reflex as indicators of injury

severity³⁵. Sham animals were connected to the FPI device, but the pendulum was not released. The injury-hub assembly was removed *en bloc*, integrity of the dura was observed, and bleeding was controlled prior to the incision being stapled. Brain-injured animals had righting reflex recovery times of 6 to 10 minutes, indicating a moderate injury severity; sham-injured animals recovered within 15 seconds. Surgical recovery was monitored post-operatively for three days and no overt differences (e.g. weight, movement, grooming) were observed between animals.

2.2 Brain preparation for Aminocupric silver technique

Argyrophilic reaction product, which has come to indicate neuropathology, was examined using the de Olmos aminocupric silver histochemical technique as previously described^{26,37,38}. Sham ($n = 3$) or brain-injured rats ($n = 3$ per time point; 1d, 2d, and 7d) were given an overdose of sodium pentobarbital (200 mg/kg i.p.) and transcardially perfused with 0.9% sodium chloride, followed by a fixative solution containing 4% paraformaldehyde and 4% sucrose in 0.1 M phosphate buffered saline (PBS). Following decapitation, the heads were stored in a fixative solution containing 15% sucrose for 24 hr, after which the brains were removed, placed in fresh fixative, and shipped for histological processing to Neuroscience Associates Inc. (Knoxville, TN). The rat brains were embedded into a single gelatin block (Multiblock Technology™; Neuroscience Associates Inc.), cut at 40 μm , stained with the de Olmos aminocupric silver technique according to proprietary protocols (Neuroscience Associates Inc.), counterstained with Neutral Red, and cover-slipped. Qualitative analysis of silver staining was conducted on photomicrographs which were acquired on a Zeiss Imager A2 microscope with AxioCam MRc5 digital camera. All three regions of interest (ROIs) were analysed at 20x magnification.

2.3 Brain preparation for vascular casting and blood brain barrier disruption

On 1d, 2d, or 7d post-injury or sham-injury, a separate cohort of animals ($n = 3/\text{group}$) were transcardially perfused with heparinized (100–200 U/ml) 0.9% saline followed by 10% neutral buffered formalin (NBF). Then the vascular imaging reagent AltaBlu™ (Numira Biosciences, Salt Lake City, UT), warmed to 50°C was perfused as per the manufacturer instructions. The perfused animal head was immersed in cold 10% NBF and stored in the refrigerator overnight to allow the AltaBlu to cure. The brains were then removed, placed in 10% NBF, and sent to Numira Biosciences (Salt Lake City, UT) for MicroCT vasculature imaging. Atlas registration was performed on each brain based on MicroCT data. Atlas data³⁹ were loaded for the whole brain, as well as for the regions of interest (ROI: motor cortex (M2) region directly underneath the injury site; primary somatosensory barrel field (S1BF), and ventral posteromedial nucleus (VPM) associated with our prior work demonstrating both pathology and functional changes^{23,26,33}; Figure 1C). Using the data from the MicroCT, each section of the brain was masked and the boundaries automatically detected. Sampled points from the boundaries of the MicroCT data and atlas template were used as control points for landmark-based registration. A transformation of the MicroCT image, using a second order polynomial transformation, was applied to the atlas ROI. A 3D ROI was produced based on the interpolation between the ROI slices as seen in Figure 1A and B. High resolution vasculature imaging was used to extract the average volume, surface

area, radius, branching, and tortuosity (curvature or twisting) of the vessels in the three selected ROIs.

2.4 Statistical analysis

Statistical analyses on quantitative vascular data were performed using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA). One-way analysis of variance (ANOVA) was used to compare averages for sham (7d) and injured (1d, 2d, and 7d) animals on each measure of vascular morphology: volume, surface area, radius, branching, and tortuosity. Tukey's HSD post-hoc analysis was performed where appropriate. Significance was set at $p < 0.05$.

3. Results

3.1 Neuropathology evidenced by argyrophilic staining

The amino-cupric de Olmos silver staining technique was used to identify neuropathology in brain regions of interest. Limited silver accumulation was noted in the sham animals, as seen in figure 2. The M2 region, located directly under the fluid pulse, did not show substantial argyrophilic reaction product at any of the three post-injury time points in brain-injured animals, as seen in figure 2. However, silver accumulation was present in the S1BF at all post-injury time points, with the greatest staining intensity observed at 7d post-injury. Consistent silver accumulation was seen in the VPM region at 1d and continued through 7d.

3.2 Acute change only in M2 vascular morphology after diffuse TBI

Uninjured and brain-injured animals were perfused with AltaBlu™ at 3 different time points post-injury to investigate vascular changes after diffuse brain injury. Vasculature was visualized and reconstructed via microCT. A one-way ANOVA was used to evaluate differences between sham and brain-injured groups for each measure of vascular morphology (average volume, surface area, radius, branching, and tortuosity) for each ROI, as shown in figures 3 and 4. Among the three ROI, only the M2 had significant injury-induced alterations to vascular morphology, and only in two of the five outcome measures: volume and surface area.

Average vessel volume in M2 was significantly increased at 1d compared to sham, 2d, and 7d groups ($F(3, 8) = 8.551$, $p = 0.0071$; Figure 3). There were no significant differences among sham, 2d and 7d time points. Changes in vessel volume were not observed in the S1BF ($F(3, 8) = 2.775$, $p = 0.1104$). There was an increase in vessel volume in the VPM, however, this was not statistically significant ($F(3, 8) = 2.955$, $p = 0.0573$). Average vessel surface area was significantly increased in M2 at 1d post-injury compared to sham, 2d, and 7d ($F(3, 8) = 7.317$, $p = 0.0111$) groups. No other comparisons were significant. No significant changes in surface area were found in the S1BF ($F(3, 8) = 2.092$, $p = 0.1796$) or VPM ($F(3, 8) = 1.168$, $p = 0.3806$; Figure 3).

For the average radius, average branching and average tortuosity, no significant differences were detected, as shown in table 1. In the M2 region, there was no significant difference between groups in the average radius ($F(3,8) = 2.506$, $p = 0.1329$), branching ($F(3,8) = 0.9660$, $p = 0.4547$), or tortuosity ($F(3,8) = 0.3423$, $p = 0.7957$). In the S1BF, there was no

significant difference between groups in the average radius ($F(3,8) = 1.368$, $p = 0.3205$), branching ($F(3,8) = 0.9212$, $p = 0.1586$), or tortuosity ($F(3,8) = 0.8992$, $p = 0.4827$). Similarly in the VPM, there was no significant difference between groups in the average radius ($F(3,8) = 0.7480$, $p = 0.5533$), branching ($F(3,8) = 1.569$, $p = 0.2711$), or tortuosity ($F(3,8) = 2.284$, $p = 0.1558$).

4. Discussion

Clinically, disturbances to the vasculature are routinely noted following TBI. PET studies have allowed investigation into vascular dilation, cerebral blood flow as well as cerebral blood volume⁴⁰. Changes to the vasculature are associated with cerebral oedema and poor outcome¹³. Limited research has focused on vascular changes following diffuse brain injury. In this study, we examined morphological characteristics of the vasculature in three brain regions following diffuse TBI in the rat. The S1BF and VPM were selected based on demonstrated neuropathology corresponding to impairments in functional performance^{25–30}, whereas the M2 was selected due to its location directly underneath the injury site. The primary aim was to determine if TBI-induced neuropathology, as evidenced by silver accumulation, was accompanied by alterations in vascular morphology. Contrary to our hypothesis, neuropathology was not associated with morphological changes in vasculature.

The primary injury associated with TBI initiates complex secondary injury cascades, which further exacerbate the initial injury. Secondary injury cascades include BBB permeability, inflammatory cascades, release of reactive oxygen species, neuropathology as well as oedema^{19,41–44}. With increased permeability of vasculature and the breakdown of the BBB, vasogenic oedema occurs. As the secondary injury propagates, cellular metabolism is impaired leading to the disruption of sodium and potassium pumps, leading to cellular retention of sodium and water, or cytotoxic oedema^{45,46}. These factors culminate in neuropathology. Silver-impregnation allows visualization of neuropathology³⁸. Consistent with previous findings, the argyrophilic reaction product of silver staining has been quantified in the S1BF, with peak accumulation occurring at 7 days post-injury²⁶, a region with astrocyte and microglia activation^{28,30,34}. Moreover, silver accumulation has been documented to mirror FluoroJade staining, another marker for neuropathology, in both rats and mice following controlled cortical impact⁴⁷. Therefore, in this study silver staining was used to indicate injury-induced neuropathology. Silver accumulation was observed over 7 days post-injury, particularly evident in the S1BF and to some extent the VPM. Compared to the S1BF and VPM, little evidence for neuropathology was observed in the M2 region directly beneath the injury site. The multifocal, diffuse nature of midline FPI is evidenced in the regional distribution of neuropathology, however the presence distal to the injury site remains a unique feature of this experimental model.

The vascular system is more heterogeneous in brain tissue than in other organs, with vessels possessing endothelial cell tight junctions that prevent or considerably hinder the influx of water-soluble molecules⁴⁸. In rats, cerebral arteries and veins almost never run in pairs, although they connect through a complex network of capillaries⁴⁸. The rat cerebrovascular system lacks direct communication between arteries and veins (A-V shunts), but retains

arterial and venous anastomoses, which create considerable redundancy and precludes complete localized ischemia of the brain⁴⁸. In the wake of diffuse brain injury, complete occlusion of vessels is unlikely and has yet to be documented, indicating that experimental TBI is discrete from experimental ischemia, despite shared cellular mechanisms. Unfortunately, the technique used in this study cannot distinguish arteries from veins and may not have been able to label capillaries exhaustively. However, it was able to show gross changes in the M2 vasculature following diffuse brain injury. These data therefore indicate further study is warranted to more thoroughly investigate vascular changes in veins, arteries and capillaries.

Reduced cerebral blood flow has been established as a component of contusion and pericontusional brain injury in the clinical population⁴⁰. In the clinical population, the decreased blood flow in the contusion area occurred in the absence of changes to cerebral blood volume. However, in the pericontusional area reduced blood flow was coupled with reduced blood volume⁴⁰. Taken together these data suggest changes to the vasculature, such as engorgement, can occur following brain injury. In the current study high resolution imaging and volumetric analysis was performed on the vascular casts in the M2, S1BF, and VPM regions at each post-injury time point. We expected to find evidence of injury-induced alterations in vascular morphology in regions with positive silver staining. For these reasons, the present approach was undertaken to observe gross changes in angiogenesis or vascular pruning, in addition to regional changes in vascular morphology. However, the only changes in vasculature were found at the earliest time point in the M2 region. Quantitative analysis of the vasculature at 1d post-FPI showed transient and significant increases in vessel volume and surface area that returned to sham levels by 2d and remained unchanged at 7d. One possible reason for the findings could be related to the proximity of M2 to the injury site. Others have demonstrated reduced vascular density in the ipsilateral cortex 1d post-lateral FPI²⁰, which returned to uninjured levels by 2 weeks post-injury, suggesting injury-related vascular remodelling. Additionally, arterial vessels viewed through a cranial window adjacent to the injury site have been reported to dilate after midline FPI in the cat^{49,50}, where vasodilation persisted through the 8-hour observation period. Increased vessel dilation could be directly related to the increase in volume and surface area observed in the present study. Of note, none of the vascular casts indicated overt haemorrhage or pooling of blood, even at the 1d post-injury time point, suggesting that any compromise to the BBB would be microscopic.

Delayed neuropathology may be secondary to vascular changes, such as cerebral blood flow and vessel occlusion. Reduced cerebral blood flow is a feature of experimental diffuse brain injury, with reports suggesting that following FPI there is a 40–50% reduction in cerebral blood flow which lasts approximately 4 hours⁵¹. For that study, 4 hours post-injury was the latest time point examined; however, in areas of decreased blood flow, neuropathology and vascular alterations are likely to follow. Regional cerebral blood flow after lateral FPI recovered to near normal rates by 2 hours post-injury, everywhere but the injury site⁵¹. This prolonged oligemia at the injury was associated with development of cystic necrosis at the injury site 4 weeks after injury⁵¹. In the present study, no changes in vascular morphology were observed concomitant with neuropathology, since the time points chosen for analysis

may be too late for the acute changes (2 hours) and too early for the chronic alterations (4 weeks).

Immediately following midline FPI in the cat, the pial arterioles dilate among other morphological and functional vascular abnormalities⁵². The authors reported the vascular changes to be mediated by the generation of free radicals. Free radicals are known to propagate secondary injury cascades leading to neuropathology. In the current study, changes in vessel function were not assessed, however the same processes which contributed to silver accumulation (neuropathology) may equally impact vessel function. Moreover, vascular dysfunction in terms of altered vascular reactivity to vasodilators has also been reported^{53,54}. These studies show altered vascular function for weeks post-injury, suggesting future studies need to examine vasoreactivity and functional changes in both the acute (<12 hours) and chronic phases post-injury. The relatively acute resolution of changes in vascular structure outlined in the current communication do not undermine the potential for long term functional vascular changes. Indeed, such vascular impairment is hypothesized to play a role in the brain's increased vulnerability to secondary insults after TBI as the impaired vessels cannot adequately respond to additional challenges. Moreover, recent clinical data indicate that cerebral blood flow may serve as a marker for enduring neurological impairments after concussion⁵⁵.

The current study assessed gross changes in vasculature via resin casting. This method may only detect gross changes with microscopic alterations being obscured by the 3D reconstruction procedures. Others have used microcorrosion casts in different cortical regions of human tissue to examine perivascular changes resulting from TBI⁴⁶. Their immunohistochemical analysis of endothelial markers revealed loss of smooth normal endothelium. For the present study, regional analysis focused on areas of known neuropathology, given our hypothesis that underlying cellular processes would most likely contribute to gross vascular change; yet, no changes were evident. Further studies could pursue vascular changes at the microscopic level and incorporate functional assessments.

5. Conclusion

The acute increase in average volume and surface area among vessels in the M2 region likely resulted from mechanical injury associated with proximity to the primary insult. However, the morphological vascular response was transient, having recovered by 2d post-injury. Comparison of the vessel volume and surface area within S1BF and VPM across all time points failed to show any TBI-induced effects. These preliminary findings suggest that morphological changes within the vascular network likely represent an acute response to the injury, rather than delayed or chronic pathological processes. Further studies investigating injury severity and type would help decipher these injury processes. More detailed analysis of vascular networks may reveal subtle neovascularization and verify reduced vasoreactivity.

Acknowledgments

The animals for this study were prepared at the University of Kentucky. The authors wish to thank Amanda Lisembee for the preparation of the animals and Dr. Pooja Talauliker for investigating the vascular casting techniques.

References

1. Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. *J Head Trauma Rehabil.* 2006; 21(5):375–378. [PubMed: 16983222]
2. Reilly P. The impact of neurotrauma on society: an international perspective. *Prog Brain Res.* 2007; 161:3–9. [PubMed: 17618966]
3. Albensi BC, Janigro D. Traumatic brain injury and its effects on synaptic plasticity. *Brain Inj.* 2003; 17(8):653–63. [PubMed: 12850950]
4. Masel BE, DeWitt DS. Traumatic brain injury: a disease process, not an event. *J Neurotrauma.* 2010; 27(8):1529–40. [PubMed: 20504161]
5. Yeates KO, Taylor HG, Wade SL, Drotar D, Stancin T, Minich N. A prospective study of short- and long-term neuropsychological outcomes after traumatic brain injury in children. *Neuropsychology.* 2002; 16(4):514–23. [PubMed: 12382990]
6. Yeates KO, Taylor HG, Woodrome SE, Wade SL, Stancin T, Drotar D. Race as a moderator of parent and family outcomes following pediatric traumatic brain injury. *J Pediatr Psychol.* 2002; 27(4):393–403. [PubMed: 11986362]
7. Stanimirovic DB, Friedman A. Pathophysiology of the neurovascular unit: disease cause or consequence? *J Cereb Blood Flow Metab.* 2012; 32(7):1207–21. [PubMed: 22395208]
8. Thornton E, Ziebell JM, Leonard AV, Vink R. Kinin receptor antagonists as potential neuroprotective agents in central nervous system injury. *Molecules.* 2010; 15(9):6598–618. [PubMed: 20877247]
9. Turner RJ, Dasilva KW, O'Connor C, van den Heuvel C, Vink R. Magnesium gluconate offers no more protection than magnesium sulphate following diffuse traumatic brain injury in rats. *J Am Coll Nutr.* 2004; 23(5):541S–544S. [PubMed: 15466961]
10. Vink R, Nimmo AJ. Multifunctional drugs for head injury. *Neurotherapeutics.* 2009; 6(1):28–42. [PubMed: 19110197]
11. Vink R, van den Heuvel C. Substance P antagonists as a therapeutic approach to improving outcome following traumatic brain injury. *Neurotherapeutics.* 2010; 7(1):74–80. [PubMed: 20129499]
12. Ziebell JM, Bye N, Semple BD, Kossmann T, Morganti-Kossmann MC. Attenuated neurological deficit, cell death and lesion volume in Fas-mutant mice is associated with altered neuroinflammation following traumatic brain injury. *Brain Res.* 2011; 1414:94–105. [PubMed: 21871613]
13. Werner C, Engelhard K. Pathophysiology of traumatic brain injury. *Br J Anaesth.* 2007; 99(1):4–9. [PubMed: 17573392]
14. Dixon CE, Lyeth BG, Povlishock JT, Findling RL, Hamm RJ, Marmarou A, Young HF, Hayes RL. A fluid percussion model of experimental brain injury in the rat. *J Neurosurg.* 1987; 67(1):110–9. [PubMed: 3598659]
15. Yuan XQ, Prough DS, Smith TL, Dewitt DS. The effects of traumatic brain injury on regional cerebral blood flow in rats. *J Neurotrauma.* 1988; 5(4):289–301. [PubMed: 3249308]
16. Unterberg AW, Stover J, Kress B, Kiening KL. Edema and brain trauma. *Neuroscience.* 2004; 129(4):1021–9. [PubMed: 15561417]
17. Schmidt RH, Grady MS. Regional patterns of blood-brain barrier breakdown following central and lateral fluid percussion injury in rodents. *J Neurotrauma.* 1993; 10(4):415–30. [PubMed: 8145265]
18. Barzo P, Marmarou A, Fatouros P, Corwin F, Dunbar J. Magnetic resonance imaging-monitored acute blood-brain barrier changes in experimental traumatic brain injury. *J Neurosurg.* 1996; 85(6):1113–21. [PubMed: 8929504]
19. Povlishock JT, Becker DP, Sullivan HG, Miller JD. Vascular permeability alterations to horseradish peroxidase in experimental brain injury. *Brain Res.* 1978; 153(2):223–39. [PubMed: 687980]
20. Park E, Bell JD, Siddiq IP, Baker AJ. An analysis of regional microvascular loss and recovery following two grades of fluid percussion trauma: a role for hypoxia-inducible factors in traumatic brain injury. *J Cereb Blood Flow Metab.* 2009; 29(3):575–84. [PubMed: 19088740]

21. Busl KM, Greer DM. Hypoxic-ischemic brain injury: pathophysiology, neuropathology and mechanisms. *NeuroRehabilitation*. 2010; 26(1):5–13. [PubMed: 20130351]
22. Kelley BJ, Farkas O, Lifshitz J, Povlishock JT. Traumatic axonal injury in the perisomatic domain triggers ultrarapid secondary axotomy and Wallerian degeneration. *Exp Neurol*. 2006; 198(2):350–60. [PubMed: 16448652]
23. Lifshitz J, Kelley BJ, Povlishock JT. Perisomatic thalamic axotomy after diffuse traumatic brain injury is associated with atrophy rather than cell death. *J Neuropathol Exp Neurol*. 2007; 66(3): 218–29. [PubMed: 17356383]
24. Singleton RH, Zhu J, Stone JR, Povlishock JT. Traumatically induced axotomy adjacent to the soma does not result in acute neuronal death. *J Neurosci*. 2002; 22(3):791–802. [PubMed: 11826109]
25. Learoyd AE, Lifshitz J. Comparison of rat sensory behavioral tasks to detect somatosensory morbidity after diffuse brain-injury. *Behav Brain Res*. 2012; 226(1):197–204. [PubMed: 21939691]
26. Lifshitz J, Lisembee AM. Neurodegeneration in the somatosensory cortex after experimental diffuse brain injury. *Brain Struct Funct*. 2012; 217(1):49–61. [PubMed: 21597967]
27. McNamara KC, Lisembee AM, Lifshitz J. The whisker nuisance task identifies a late-onset, persistent sensory sensitivity in diffuse brain-injured rats. *J Neurotrauma*. 2010; 27(4):695–706. [PubMed: 20067394]
28. Taylor SE, Morganti-Kossmann C, Lifshitz J, Ziebell JM. Rod microglia: a morphological definition. *PLoS One*. 2014; 9(5):e97096. [PubMed: 24830807]
29. Thomas TC, Hinzman JM, Gerhardt GA, Lifshitz J. Hypersensitive glutamate signaling correlates with the development of late-onset behavioral morbidity in diffuse brain-injured circuitry. *J Neurotrauma*. 2012; 29(2):187–200. [PubMed: 21939393]
30. Ziebell JM, Taylor SE, Cao T, Harrison JL, Lifshitz J. Rod microglia: elongation, alignment, and coupling to form trains across the somatosensory cortex after experimental diffuse brain injury. *J Neuroinflammation*. 2012; 9:247. [PubMed: 23111107]
31. Dietrich WD, Ginsberg MD, Busto R, Smith DW. Metabolic alterations in rat somatosensory cortex following unilateral vibrissal removal. *J Neurosci*. 1985; 5(4):874–80. [PubMed: 3981247]
32. Petersen CC. Cortical control of whisker movement. *Annu Rev Neurosci*. 2014; 37:183–203. [PubMed: 24821429]
33. Hall KD, Lifshitz J. Diffuse traumatic brain injury initially attenuates and later expands activation of the rat somatosensory whisker circuit concomitant with neuroplastic responses. *Brain Res*. 2010; 1323:161–73. [PubMed: 20122903]
34. Cao T, Thomas TC, Ziebell JM, Pauly JR, Lifshitz J. Morphological and genetic activation of microglia after diffuse traumatic brain injury in the rat. *Neuroscience*. 2012; 225:65–75. [PubMed: 22960311]
35. Hosseini AH, Lifshitz J. Brain injury forces of moderate magnitude elicit the fencing response. *Med Sci Sports Exerc*. 2009; 41(9):1687–97. [PubMed: 19657303]
36. Kelley BJ, Lifshitz J, Povlishock JT. Neuroinflammatory responses after experimental diffuse traumatic brain injury. *J Neuropathol Exp Neurol*. 2007; 66(11):989–1001. [PubMed: 17984681]
37. de Olmos JS, Beltramino CA, de Olmos de Lorenzo S. Use of an amino-cupric-silver technique for the detection of early and semiacute neuronal degeneration caused by neurotoxicants, hypoxia, and physical trauma. *Neurotoxicol Teratol*. 1994; 16(6):545–61. [PubMed: 7532272]
38. Switzer RC 3rd. Application of silver degeneration stains for neurotoxicity testing. *Toxicol Pathol*. 2000; 28(1):70–83. [PubMed: 10668992]
39. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Amsterdam: Academic Press/Elsevier; 2007.
40. Hattori N, Huang SC, Wu HM, Liao W, Glenn TC, Vespa PM, Phelps ME, Hovda DA, Bergsneider M. PET investigation of post-traumatic cerebral blood volume and blood flow. *Acta Neurochir Suppl*. 2003; 86:49–52. [PubMed: 14753403]
41. Hellewell SC, Yan EB, Agyapomaa DA, Bye N, Morganti-Kossmann MC. Post-traumatic hypoxia exacerbates brain tissue damage: analysis of axonal injury and glial responses. *J Neurotrauma*. 2010; 27(11):1997–2010. [PubMed: 20822466]

42. Vink R, Mullins PG, Temple MD, Bao W, Faden AI. Small shifts in craniotomy position in the lateral fluid percussion injury model are associated with differential lesion development. *J Neurotrauma*. 2001; 18(8):839–47. [PubMed: 11526990]
43. Vink R, Young A, Bennett CJ, Hu X, Connor CO, Cernak I, Nimmo AJ. Neuropeptide release influences brain edema formation after diffuse traumatic brain injury. *Acta Neurochir Suppl*. 2003; 86:257–60. [PubMed: 14753447]
44. Ziebell JM, Morganti-Kossmann MC. Involvement of pro- and anti-inflammatory cytokines and chemokines in the pathophysiology of traumatic brain injury. *Neurotherapeutics*. 2010; 7(1):22–30. [PubMed: 20129494]
45. Giza CC, Hovda DA. The Neurometabolic Cascade of Concussion. *J Athl Train*. 2001; 36(3):228–235. [PubMed: 12937489]
46. Rodriguez-Baeza A, Reina-de la Torre F, Poca A, Marti M, Garnacho A. Morphological features in human cortical brain microvessels after head injury: a three-dimensional and immunocytochemical study. *Anat Rec A Discov Mol Cell Evol Biol*. 2003; 273(1):583–93. [PubMed: 12808643]
47. Hall ED, Bryant YD, Cho W, Sullivan PG. Evolution of post-traumatic neurodegeneration after controlled cortical impact traumatic brain injury in mice and rats as assessed by the de Olmos silver and fluorojade staining methods. *J Neurotrauma*. 2008; 25(3):235–47. [PubMed: 18352837]
48. Scremin, OU. Cerebral Vascular System. In: Paxinos, G., editor. *The Rat Nervous System*. USA: Elsevier Academic Press; 2004. p. 1165-1202.
49. Kontos HA, Wei EP, Ellis EF, Dietrich WD, Povlishock JT. Prostaglandins in physiological and in certain pathological responses of the cerebral circulation. *Fed Proc*. 1981; 40(8):2326–30. [PubMed: 7238914]
50. Wei EP, Dietrich WD, Povlishock JT, Navari RM, Kontos HA. Functional, morphological, and metabolic abnormalities of the cerebral microcirculation after concussive brain injury in cats. *Circ Res*. 1980; 46(1):37–47. [PubMed: 7349916]
51. Yamakami I, McIntosh TK. Effects of traumatic brain injury on regional cerebral blood flow in rats as measured with radiolabeled microspheres. *J Cereb Blood Flow Metab*. 1989; 9(1):117–24. [PubMed: 2910893]
52. Povlishock JT, Kontos HA. Continuing axonal and vascular change following experimental brain trauma. *Cent Nerv Syst Trauma*. 1985; 2(4):285–98. [PubMed: 3836013]
53. Baranova AI, Wei EP, Ueda Y, Sholley MM, Kontos HA, Povlishock JT. Cerebral vascular responsiveness after experimental traumatic brain injury: the beneficial effects of delayed hypothermia combined with superoxide dismutase administration. *J Neurosurg*. 2008; 109(3):502–9. [PubMed: 18759584]
54. Wei EP, Hamm RJ, Baranova AI, Povlishock JT. The long-term microvascular and behavioral consequences of experimental traumatic brain injury after hypothermic intervention. *J Neurotrauma*. 2009; 26(4):527–37. [PubMed: 19245307]
55. Meier TB, Bellgowan PS, Singh R, Kuplicki R, Polanski DW, Mayer AR. Recovery of Cerebral Blood Flow Following Sports-Related Concussion. *JAMA Neurol*. 2015

Figure 1.

Neurovascular imaging and regions of interest. Atlas registration was performed on each brain included in the vascular casting study. Registration was accomplished using MicroCT and an atlas template to identify anatomical landmarks. A 3D region of interest (ROI) was produced based on the interpolation between the ROI on individual sections/slices. (A, B) Axial and sagittal images, respectively, from a 3D model showing each ROI, based on atlas registration of the M2 (light blue), S1BF (red), and VPM (green). Analyses and imaging was performed by Numira Biosciences (Salt Lake City, UT). (C) Figure from *The Rat Brain in Stereotaxic Coordinates*, Paxinos and Watson (2007) showing a coronal view of ROIs using the same color scheme as the 3D images. Secondary motor cortex (M2); primary somatosensory barrel field (S1BF); ventral posteromedial nucleus of the thalamus (VPM); region of interest (ROI).

Figure 2.

De Olmos silver stain shows region-specific neuropathology. Deposits of aminocupric staining are indicative of neuropathology and show a regional and temporal pattern following diffuse TBI. As expected, no staining was observed in the sham animals. The M2 did not show evidence of neuropathology at 1d, 2d or 7d after fluid percussion injury (FPI). The S1BF and the thalamus, including the VPM, had silver stain visible at 1d which spread more diffusely over time post-injury and the intensity of staining increased over 2d and 7d. Dark and intense staining in S1BF and VPM suggests neuropathology and circuit disruption.

Figure 3.

Changes in neurovascular volume and surface area following diffuse TBI. Analysis of the vasculature in the secondary motor cortex (M2) region showed significant differences in vessel volume and surface area. Tukey's HSD post hoc analysis revealed that both vessel volume and surface area were significantly increased in M2 at 1d after fluid percussion injury (FPI) relative to 2d, 7d and sham. There were no significant changes observed in vessel volume or surface area between 2d and 7d post-injury relative to sham or each other in M2. In the primary somatosensory barrel field cortex (S1BF) there were no significant changes in vessel volume or surface area ($p > 0.05$). Analysis of the ventral posteromedial thalamus (VPM) also failed to show any significant differences in vessel volume ($p = 0.0573$) or surface area ($p = 0.3806$). However, there was a similar, but non-significant, pattern of vascular change in vessel volume and surface area in the S1BF at 1d, 2d, and 7d post-injury. Data are presented as the mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$.

Table 1

Changes in vessel radius, branch number and tortuosity were not evident following injury. Neither diffuse brain injury (fluid percussion injury, FPI) nor sham-injury produced significant changes in the average vessel radius, branching or tortuosity at any of the post-injury time points in M2, S1BF or VPM ($p > 0.05$). Data are presented as the mean $\pm$ SEM.

Average radius, branching and tortuosity of vasculature in the M2, S1BF and VPM within the first week post-injury.

	Sham			1d FPI			2d FPI			7d FPI		
	M2	S1BF	VPM	M2	S1BF	VPM	M2	S1BF	VPM	M2	S1BF	VPM
Average radius (μm)	27.34 $\pm$ 0.8	28.41 $\pm$ 2.2	29.05 $\pm$ 2.4	27.34 $\pm$ 0.8	29.66 $\pm$ 0.7	28.07 $\pm$ 1.7	35.23 $\pm$ 4.6	33.67 $\pm$ 3.5	29.53 $\pm$ 1.5	27.09 $\pm$ 1.7	28.26 $\pm$ 1.0	26.23 $\pm$ 0.8
Average branching (vessel branches/mm)	1.48 $\pm$ 0.1	1.49 $\pm$ 0.1	1.20 $\pm$ 0.2	1.53 $\pm$ 0.04	1.50 $\pm$ 0.11	1.41 $\pm$ 0.1	1.40 $\pm$ 0.02	1.57 $\pm$ 0.1	1.77 $\pm$ 0.3	1.37 $\pm$ 0.1	1.45 $\pm$ 0.15	1.329 $\pm$ 0.06
Average tortuosity	1.32 $\pm$ 0.03	1.29 $\pm$ 0.03	1.303 $\pm$ 0.06246	1.31 $\pm$ 0.02	1.29 $\pm$ 0.03	1.33 $\pm$ 0.02	1.30 $\pm$ 0.02	1.34 $\pm$ 0.02	1.44 $\pm$ 0.04	1.30 $\pm$ 0.03	1.29 $\pm$ 0.01	1.34 $\pm$ 0.004