Evidence of early vasogenic edema following minor head impact that can be reduced with a vasopressin V1a receptor antagonist

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Abstract

Background: Does minor head impact without signs of structural brain damage cause short-term changes in vasogenic edema as measured by an increase apparent diffusion coefficient (ADC) using diffusion weighted imaging? If so, could the increase in vasogenic edema be treated with a vasopressin V1a receptor antagonist? We hypothesized that SRX251, a highly selective V1a antagonist, would reduce vasogenic edema in response to a single minor head impact.

Methods: Lightly anesthetized male rats were subjected to a sham procedure or a single hit to the forehead using a closed skull, momentum exchange model. Animals recovered in five min and were injected with saline vehicle (n=8) or SRX251 (n=8) at 15 min post head impact and again 7–8 hrs later. At 2 hrs, 6 hrs, and 24 hrs post injury, rats were anesthetized and scanned for increases in ADC, a neurological measure of vasogenic edema. Sham rats (n=6) were exposed to anesthesia and scanned at all time points but were not hit or treated. Images were registered to and analyzed

using a 3D MRI rat atlas providing site-specific data on 150 different brain areas. These brain areas were parsed into 11 major brain regions.

Results: Untreated rats with brain injury showed a significant increase in global brain vasogenic edema as compared to sham and SRX251 treated rats. Edema peaked at 6 hrs in injured, untreated rats in three brain regions where changes in ADC were observed, but returned to sham levels by 24 hrs. There were regional variations in the time course of vasogenic edema and drug efficacy. Edema was significantly reduced in cerebellum and thalamus with SRX251 treatment while the basal ganglia did not show a response to treatment.

Conclusion: A single minor impact to the forehead causes regional increases in vasogenic edema that peak at 6 hrs but return to baseline within a day in a subset of brain regions. Treatment with a selective V1a receptor antagonist can reduce much of the edema.

Keywords

traumatic brain injury; diffusion weighted imaging; apparent diffusion coefficient; magnetic resonance imaging; cerebellum

Background

Traumatic brain injury (TBI) affects more than 50 million people each year worldwide across all demographic and socioeconomic groups [39]. In the United States and Europe, TBI accounts for over 4 million emergency hospital visits annually [39, 40, 69]. Major traumatic brain injuries are a contributing factor to a third of injury-related deaths in the United States and is one of the leading causes of death and disability in persons under 35 and over 65 [69]. In the United States alone, the annual economic burden of TBI is conservatively estimated at $86 billion [39]. While these statistics point to the significance of TBI as a health issue, one form of brain injury, the effects of a single, minor impact that does not require hospitalization or necessitate immediate medical attention, are not well understood [10]. The health community lists guidelines for diagnosing these "mild" head injuries that include self-reports of transient confusion, disorientation, impaired consciousness, or dysfunction in memory around the time of the injury and, importantly, no apparent structural damage as determined with imaging [1, 10, 65]. The self-reports invite a "bump on the head, ice pack" approach to the problem.

There are neurological complications following any closed head injury, including mild TBI [30, 70]. Edema is among the most significant complications because it is a critical component in the pathophysiology of brain injury and occurs in stages. Vasogenic edema is caused by disruption of the blood-brain barrier (BBB) and, in moderate and severe TBI, is followed by cytogenic edema from cellular swelling and lastly, by a combination of both [28]. The timing and severity of each is dependent upon the level of brain injury. The extent of edema occurring after TBI can be determined by measures of apparent diffusion coefficient (ADC) using diffusion weighted imaging (DWI) [70]. ADC is a quantitative measure of water mobility. Cellular swelling due to loss of homeostatic regulation of osmolarity across the plasma membrane results in lower ADC values. Vasogenic edema, characterized by higher ADC values, results from a change in permeability across the BBB,

with an influx of water and solute into the interstitial space contributing to the extracellular volume. This increase in brain water contributes to parenchymal swelling and increase in intracranial pressure.

There are currently no approved pharmaceutical treatments that can prevent, minimize, or reverse brain edema following any level of brain damage [17, 23]. Several labs have studied the role of the neuropeptide arginine vasopressin (AVP) in intracranial water regulation [2, 15, 18, 32, 57]. AVP is a naturally occurring neuropeptide hormone involved in brain water permeability and vasoconstriction [33, 47]. The AVP V1a (V1a) receptor subtype is highly expressed in cortical and subcortical brain areas across all mammals [52, 77] and AVP signaling through this receptor is strongly tied to the pathology of cerebral edema in TBI. For instance, V1a receptor density increases following TBI and infusion of V1a antagonists block increased intracranial pressure after brain injury in animal models [6, 20, 43]. Knockout mice lacking V1a receptors show reduced edema, secondary injuries, and behavioral impairments in the days following brain damage [55]. We recently showed that systemic treatment with a highly selective V1a receptor antagonist for 5 days starting within 24 hrs of moderate/severe brain TBI significantly reduced edema and prevented cognitive deficits [46].

The present study was designed to address two questions: 1) Does minor head impact without signs of structural brain damage cause short-term changes in vasogenic edema as measured by an increase in ADC and, if so, 2) could these changes be mitigated by treatment with an AVP receptor V1a antagonist.

Methods

Animals

Male Sprague Dawley rats (n=22) weighing between 300 and 325 gm were obtained from Charles River Laboratories (Wilmington, Massachusetts, USA). Rats were housed in Optirat® GenII (Animal Care Systems, Inc., Centennial, CO) cages with Bed-o'Cobs® corn-cob bedding (Anderson lab bedding, Delphi, IN) mixed with Envirodri™ shredded paper (Shepards Specialty Papers, Richland, MI). The vivarium was maintained at an ambient temperature of (22–24°C), humidity (35–45%) on a 12:12 light:dark cycle (lights on at 07:00 a.m.). Rats were allowed access to food and fresh water *ad libitum*. Rats were imaged during the light phase of the circadian cycle. All rats were acquired and cared for in accordance with the guidelines published in the NIH Guide for the Care and Use of Laboratory Animals. All methods and procedures described below were pre-approved by the Northeastern University Institutional Animal Care and Use Committee and adhere to the ARRIVE guidelines for reporting in vivo experiments in animal research [31]

Treatment

Lightly isoflurane anesthetized rats were subjected to a sham procedure or a single mild hit to the forehead, midline and rostral to Bregma, using a closed skull, momentum exchange model [36]. Originally developed by Viano and colleagues to reliably inflict mild, medium, or severe rat brain injury [73], this same model was further refined by Mychasiuk et al., and

used to test the behavioral effects of TBI controlling for the axis of injury, rotational force, and head acceleration in different directions [48]. All rats recovered from the sham or mild head impact within 5 min as demonstrated by righting and motor activity. There was no perioperative procedure for pain. Rats were injected with saline vehicle (n=8) or SRX251 (n=8) 15 min post head impact and again 7–8 hrs later. At 2 hrs, 6 hrs, and 24 hrs post head impact, rats were anesthetized and scanned for structural brain damage and increases in ADC, a neurological measure of vasogenic edema. Sham rats (n=6) were exposed to anesthesia and scanned at all time points but were not hit or treated. Scanning sessions lasted about 70 min, after which animals were returned to their respective home cage.

Neuroimaging

Imaging sessions were conducted using a Bruker Biospec 7.0 T/20-cm USR horizontal magnet (Bruker, Billerica, MA, USA) and a 20-G/cm magnetic field gradient insert (ID=12 cm) capable of a 120-μs rise time. Radio frequency signals were sent and received with a quadrature volume coil built into the animal restrainer (Animal Imaging Research, Holden, Massachusetts). The design of the restraining system included a padded head support obviating the need for ear bars, helping to reduce discomfort while minimizing motion artifact. All rats were imaged under 1–2% isoflurane while keeping a respiratory rate of 40–50 breathes/min. At the beginning of each imaging session, a high-resolution anatomical data set was collected for assessment of structural damage using the RARE pulse sequence with following parameters, 35 slice of 0.7mm thickness; field of view [FOV] 3 cm; 256×256; repetition time [TR] 3900 msec; effective echo time [TE] 48 msec; NEX 3; 6 min 14 sec acquisition time.

Diffusion Weighted Imaging – Quantitative Anisotropy

DWI was acquired with a spin-echo echo-planar-imaging (EPI) pulse sequence having the following parameters: TR/TE = 500/20 msec, eight EPI segments, and 10 non-collinear gradient directions with a single b-value shell at 1000 s/mm 2 and one image with a B-value of 0 s/mm 2 (referred to as B 0) [8, 19, 36]. Geometrical parameters were: 48 coronal slices, each 0.313 mm thick (brain volume) and with in-plane resolution of 0.313×0.313 mm 2 (matrix size 96×96; FOV 30 mm^3). The imaging protocol was repeated two times for signal averaging. Each DWI acquisition took 35 min and the entire MRI protocol including the anatomy lasted about 90 min. Image analysis included DWI analysis of the DWI-3D-EPI images to produce the maps of ADC. DWI analysis was completed with MATLAB and MedINRIA (1.9.0; http://www-sop.inria.fr/asclepios/software/MedINRIA/index.php) software. Because sporadic excessive breathing during DWI acquisition can lead to significant image motion artifacts that are apparent only in the slices sampled when motion occurred, each image (for each slice and each gradient direction) was screened prior to DWI analysis. If found, acquisition points with motion artifacts were eliminated from analyses.

For statistical comparisons among rats, each brain volume was registered to a 3D MRI Rat Brain Atlas (© 2012 Ekam Solutions LLC, Boston, MA) allowing voxel- and region-based statistics. All image transformations and statistical analyses were carried out using the in-house MIVA software (http://ccni.wpi.edu/). For each rat, the B 0 image was co-registered with the B 0 template (using a 6-parameter rigid-body transformation). The co-registration

parameters were then applied on the DWI ADC maps. Normalization was performed on the maps because they provided the most detailed visualization of brain structures and allowed for more accurate normalization. The normalization parameters were then smoothed with a 0.3-mm Gaussian kernel. To ensure that ADC values were not affected significantly by the pre-processing steps, the 'nearest neighbor' option was used following registration and normalization. Statistical differences in measures of DWI between experimental groups for each of 11 brain regions were determined using two-way ANOVA followed by Bonferroni post hoc tests (alpha set at 5%).

The 3D MRI rat atlas has 173 segmented, annotated brain areas. For this study, 150 areas were chosen for analysis because they could be organized into well-defined neuroanatomical regions. Areas excluded were white matter tracts because they traverse several brain regions. Circumventricular organs, e.g. anterior and posterior pituitary, pineal gland, area postrema, median eminence and small adjacent areas like the arcuate and retrochiasmatic nuclei, were also excluded because of their larger, more fenestrated blood vessels. Also excluded were areas with no clear regional organization e.g., prerubral field. The remaining 150 brain areas were divided into 11 brain regions: cerebellum (20), cortex (19), thalamus (20), basal ganglia (10), hypothalamus (14), hippocampus (9), prefrontal cortex (9), olfactory bulb/ cortex (8), amygdala (8), midbrain/pons (12), brainstem (21). The areas and their average ADC values for each experimental condition and for 2, 6 and 24 hrs, are provided in Supplementary Data Excel S1. The organization was based on conventional neuroanatomy and an effort to keep individual brain areas localized and contiguous within a region. The olfactory bulb/cortex is the exception as the piriform cortex extends some distant caudally along the ventral lateral cortex away from the bulbs.

Results

Shown in Fig 1 are box and whisker scatter plots for measures of ADC in the whole brain (150 areas) for each experimental condition over the three time points following brain injury. The horizontal line in each box denotes the median. The two-way ANOVA shows a significant main effect among treatments, time of measurement over the 24 hr period, and interaction between both. At 2 hrs post head injury, vehicle treated rats (blue box) showed a significant ($p<0.001$) increase over sham (black box) and SRX251 (red box). ADC values in injured vehicle-treated animals were still significantly greater than sham or SRX251 ($p<0.05$) at 6 hr. At the 24 hr time point, there were no significant differences among the groups in ADC values. There was no evidence of structural brain damage in the 16 rats exposed to a single mild head impact during the 24 hr study period Fig 2.

To determine which areas of the brain were most sensitive to this single mild head impact directed to the forebrain, ANOVA's with post hoc analyses were run on the 11 brain regions. Only the cerebellum, thalamus, and basal ganglia showed a significant increase in ADC between experimental groups. Fig 3 shows scatter plots for ADC measures in the cerebellum. The two-way ANOVA results and list of twenty brain areas that comprise the cerebellum taken from the rat 3D MRI atlas are shown. At 2 and 6 hrs post impact, injured vehicle-treated rats present with significantly greater edema than sham ($p<0.01$), a difference that was no longer found at 24 hrs. At 6 hrs, ADC values peak in injured vehicle animals and

significantly exceed the two hr levels (p< 0.02). SRX251 treatment significantly reduced edema at the 2 and 24 hr time points compared to vehicle (p< 0.001), but the effect was not significant at the 6 hr time point. There are no significant differences at any time point between SRX251 and sham rats.

Fig 4 shows scatter plots for ADC measures in the thalamus, the two-way ANOVA table and a list of twenty brain areas that comprise the thalamus taken from the rat 3D MRI atlas. The profile of ADC values over the 24 hr period for each of the experimental conditions are similar to the cerebellum. At 2 and 6 hrs post impact, injured vehicle-treated rats showed significantly greater edema than sham (both p's<0.001). Edema in the vehicle group peaked at 6 hrs and is significantly greater than both sham and SRX251 (p<0.001). The SRX251 treatment significantly reduced edema compared to vehicle at 2 and 6 hrs (p<0.001) and 24 h (p<0.01). There are no significant differences at any time point between SRX251 and sham rats.

Fig 5 shows scatter plots for ADC measures in the basal ganglia, the two-way ANOVA table, and a list of ten brain areas that comprise the basal ganglia taken from the rat 3D MRI atlas. The change in ADC measures at 2 hr is not significantly different among conditions. By 6 hrs, injured vehicle-treated rats show significantly greater edema than sham but not SRX251. At 24 hrs, ADC values show no significant differences between conditions. At no time point is SRX251 significantly different from sham.

The 3D reconstruction shown in Fig 6 summarizes the brain regions with significant increases in edema over the 24 hr period following impact to the forebrain (location depicted in black). The thalamus (light red) and cerebellum (dark red) were responsive to SRX251 treated and we found significantly reduced edema in these areas. While the basal ganglia only showed an increase in edema at 6 hrs, as noted above, a decrease in ADC was present in response to SRX2521 but did not differ significantly when compared to vehicle (area in blue).

Discussion

This was an initial study examining the effects of a single, minor head impact on the development of early edema in a closed head, momentum exchange model of brain injury and the ability of a V1a receptor antagonist to reduce or block observed changes. The ADC from diffusion weighted imaging was used as a proxy for vasogenic edema. In this model of mild head impact, edema was significantly increased in whole brain and a subset of the 11 regions that were tested, including basal ganglia, cerebellum, and thalamus, without evidence of structural damage. The observed edema was transient because at 24 hours post-injury, vehicle-treated rats with mild head impact did not differ from sham controls in ADC. V1a antagonist treatment significantly reduced edema compared to vehicle treatment in whole brain, thalamus, and cerebellum.

Edema is a critical component in the pathophysiology of TBI and can occur within the first few hrs post-injury [3, 68, 71]. The type of edema occurring after TBI can be determined using DWI with ADC, a quantitative measure of water mobility [3, 25, 26, 56, 61].

Vasogenic edema, characterized by higher ADC values, results from a change in permeability across the BBB with an influx of water and solute into the interstitial space contributing to the extracellular volume. This increase in brain water contributes to parenchymal swelling and an increase in intracranial pressure. In the present study, only higher ADC values were found in the affected regions, indicating that a single, mild head impact can induce vasogenic edema.

An important question is why the cerebellum and thalamus, regions caudal to the site of impact on the forehead, but not the prefrontal cortex immediately beneath the site of impact, show the greatest increases in ADC? The transmission of mechanical force down white matter tracts and along brain areas abutting the ventricular system may offer an explanation [21]. The cerebellum has been recognized as being particularly vulnerable to mTBI [44, 49, 54, 72] and neuroradiological evidence of cerebellar dysfunction has been advanced as a diagnostic biomarker of TBI [72]. Because this is the only study, we know of that used a closed-head, mild injury protocol, we cannot readily compare our findings to the other rodent models with structural brain damage.

The increase in ADC, which reflects vasogenic edema, suggests a change in the permeability of the BBB with a single mild head impact. Using extravasation of blood biomarkers, e.g. Evan blue, plasma immunoglobulins, horse radish peroxidase, and albumin, there is an increase in BBB permeability within 1–4 hr hrs after brain damage in rodent models of TBI [4, 22, 24, 67]. Similar results have been reported in rats using dynamic contrast enhanced (DCE) MRI to measure BBB permeability [38, 78]. In humans, DCE MRI shows an increase in BBB permeability around damage tissue within 24 hrs of injury that persists for days [29]. However, all the work cited above follows moderate TBI with brain damage. Athletes playing American football and thought to have mild, sub-concussive head injuries show evidence of increased BBB permeability with DCE MRI [74]. Using DCE-MRI, O'Keeffe and colleagues reported BBB disruption in response to repetitive mild concussions in rugby players after a season and mixed martial arts fighters within days after a competitive fight [51]. There were no significant differences in self-reported concussions between these players and control subjects (track and field athletes). Players with multiple sub concussive collisions in American football show elevated blood levels of astrocytic protein S100B thought to be due to BBB disruption [42]. Interestingly, professional boxers show an increase in ADC values in large white matter tracts and a general increase in brain diffusivity thought to be the cumulative effect of multiple non-severe head impacts [11, 79, 80]. To our knowledge, these are the first data reporting evidence of global and region-specific increases in brain edema to a single, mild head impact in the absence of structural brain damage.

The biology of AVP contributes to the edema associated with TBI. How this neuropeptide promotes edema remains unclear, but the evidence of its involvement is substantial. Following a moderate impact to the brain, the tissue surrounding the damage parenchyma increases the expression of V1a receptors on neurons, astrocytes and endothelium of microvessels, an effect that occurs within hrs and persists for days [53, 62]. AVP appears to have a role in post-traumatic neuroinflammation as the proinflammatory chemokines in endothelial cells and astrocytes of damaged tissue are reduced in AVP-deficient Brattleboro

rats as compared to wild-type controls [63, 64]. With brain damage, the water content from vasogenic edema goes up dramatically in the first 5 to 24 hrs [43, 68, 71]. The increase in brain edema can be reduced by the continuous intravenous infusion of SR49059, a V1a receptor antagonist, immediately after brain injury [20, 43, 68]. The intracerebroventricular injection of V1a receptor antagonists e.g., SR-49059, V1880 [35] or the peptidic deamino-Pen(1), O-Me-Tyr(2), Arg(8)]-vasopressin [34, 71] are also effective but only when given within the first few hrs of injury [35]. Moderate brain injury is associated with elevated levels of the water channel AQP4, GFAP, and V1a receptor, and disruption in sodium/potassium balance, all of which can be corrected by continuous exposure to SR49059 [20, 43, 68]. The edema in the these moderate models of TBI is reduced in V1a null mice [55]. Szczygielski and coworkers used DWI to measure changes in brain edema 24 hrs post head trauma in a closed-head mouse model of moderate TBI[60]. Impact to one side of the cortex increased AQP4 levels in remote brain areas rather than the trauma epicenter itself. Interestingly, treating the core site of brain injury with focal hypothermia reduced the remote expression of AQP4 levels. In a recent study, we reported that AVN576, an orally active V1a receptor antagonist, given ip 24 hrs post injury for 5 days, could effectively reduce edema following TBI with brain damage [46].

Limitations and Data Interpretation

This was an initial study with a limited number of animals to explore the possibility that we could measures significant changes in ADC following a single mild head impact. As such there were several limitations: 1) ADC is a proxy for vasogenic edema and while there was no evidence of structural brain damage, post mortem histology looking at site-specific or regional signs of inflammation or alterations in BBB permeability would have helped to interpret the findings [59]. 2) The sensitivity of DWI was such that it did not allow us to examine 150 individual brain areas; instead, we were only able to observe change in ADC values in the whole brain and in three brain regions, cerebellum, thalamus, and basal ganglia. 3) Would there have been sex differences if we ran female rats? We are aware of the many studies reporting sex differences in response to TBI, e.g. [5, 7, 12–14, 58, 75]. Recent reviews of the literature, report there are male/female differences in morbidity and mortality following TBI [9, 45]. However, the data collected over decades of preclinical and clinical research are inconsistent, certainly as it relates to the role of estrogen and progesterone [76]. These initial studies were not designed to address the issues surrounding hormones and head injury. 4) 5) Animals were exposed to light isoflurane anesthesia four times over a 24 hr period. This necessary condition over this abbreviated time span precluded taking any meaningful measures of motor or cognitive behavior.

These data reinforce concerns expressed by others about the long-term effect of mild brain injury [51]. For example, mild brain injury begins with an impact that by itself may be recorded but not pursued as needing medical attention. Yet here we show there are global changes in vasogenic edema presumably caused by disruption in the BBB. Newsome et al. [50] reported modest neurocognitive abnormalities and increased functional connectivity in adolescent athletes with sports related concussions as compared to age matched orthopedically injured controls. Despite these signs of altered cortical function, post-concussive symptoms were judged to be resolved and athletes could return to competition.

Indeed, more adolescent players are suffering from undetected neurological problems than previously thought [66]. A single season of high school American football produces DWI changes in diffusivity in the absence of clinical concussion [16]. Multiple mild head injuries are a risk factor for cognitive, and emotion impairment and neurodegenerative disorders in athletes [41].

Conclusion

We were interested in two primary questions 1) could we detect global changes in brain edema using ADC, and 2) could these changes be mitigated by drug treatment with a vasopressin 1a antagonist? This study differs from previous animal TBI studies as it relies on imaging, without animal behavior, to evaluate the consequences of a mild head impact. The Glasgow Coma Scale of 13–15 defines a brain injury as "mild" based on measures of motor behavior, verbal responses and eye opening, with loss of consciousness and short hospitalization [27]. With CT and MRI, physical damage to the brain can be confirmed, adjusting the classification to "mild with complication" [37]. Organized sports in adolescence added another dimension to the characterization of "mild." The Centers for Disease Control and Prevention, World Health Organization and the Veteran's Administration have included self-reports of transient confusion, disorientation, impaired consciousness or dysfunction in memory around the time of the injury - importantly *there should be no structural damage as determined with imaging* [1, 10, 65]. This study in rats used a single head impact that caused no structural damage to the brain but clearly showed global and regional increases in vasogenic edema that appear within 2 hrs, increase at 6 hrs, but return to baseline within a day. Systemic treatment with a selective V1a receptor antagonist that can cross the BBB reduces much of the vasogenic edema. These findings highlight the potential risk to a single mild head impact and the role AVP in acute vasogenic edema.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

List of Abbreviations

EPI	echo planar imaging
MRI	magnetic resonance imaging
AVP	arginine vasopressin
V1a	vasopressin V1a receptor

FOV	field of view
RARE	rapid acquisition relaxation enhanced
BBB	blood-brain barrier
ADC	apparent diffusion coefficient
DWI	diffusion weighted imaging
TBI	traumatic brain injury
DCE	dynamic contrast enhanced
TR	repetition time
TE	echo time
NEX	number of excitations

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Highlights

- A single, mild head impact causes a rise in vasogenic edema 2–6 hrs post impact.

- Areas most affected, like the cerebellum, are remote from the site of impact.

- The edema peaks within 6 hrs of head impact but resolves within 24 hrs.

- A vasopressin V1a receptor antagonist blocks much of the early vasogenic edema.
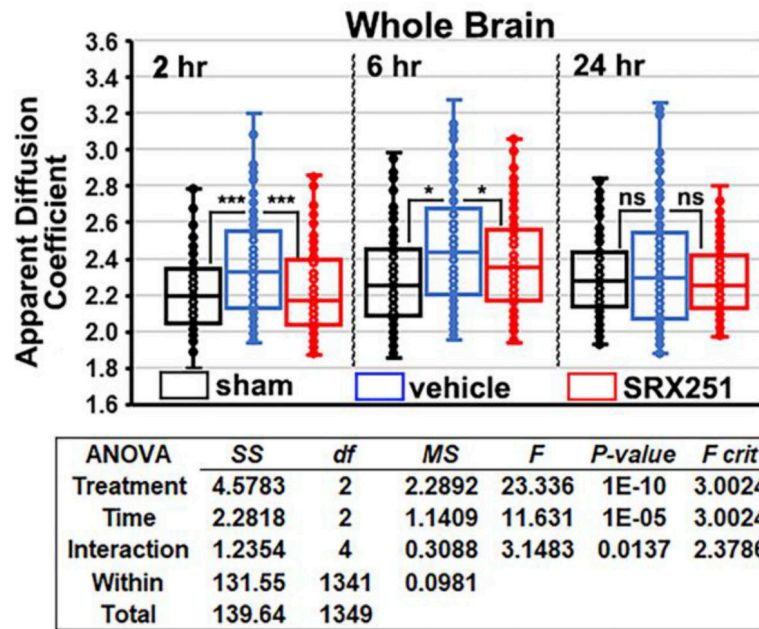
Figure 1. Edema in whole brain

Shown are scatter plots of average ADC values taken from whole brain (150 areas) for sham (black), vehicle (blue) and SRX251 (red) treatments 2, 6 and 24 hrs post head injury. The analysis from a two-way ANOVA is shown below. ns – not significant, *** p<0.001, * p<0.05

Cerebellum

ANOVA	SS	df	MS	F	P-value	F crit
Treatment	1.1476	2	0.5738	13.949	2E-06	3.0488
Time	1.3773	2	0.6887	16.741	2E-07	3.0488
Interaction	0.4556	4	0.1139	2.7687	0.029	2.4245
Within	7.0344	171	0.0411			
Total	10.015	179				

Cerebellar Areas

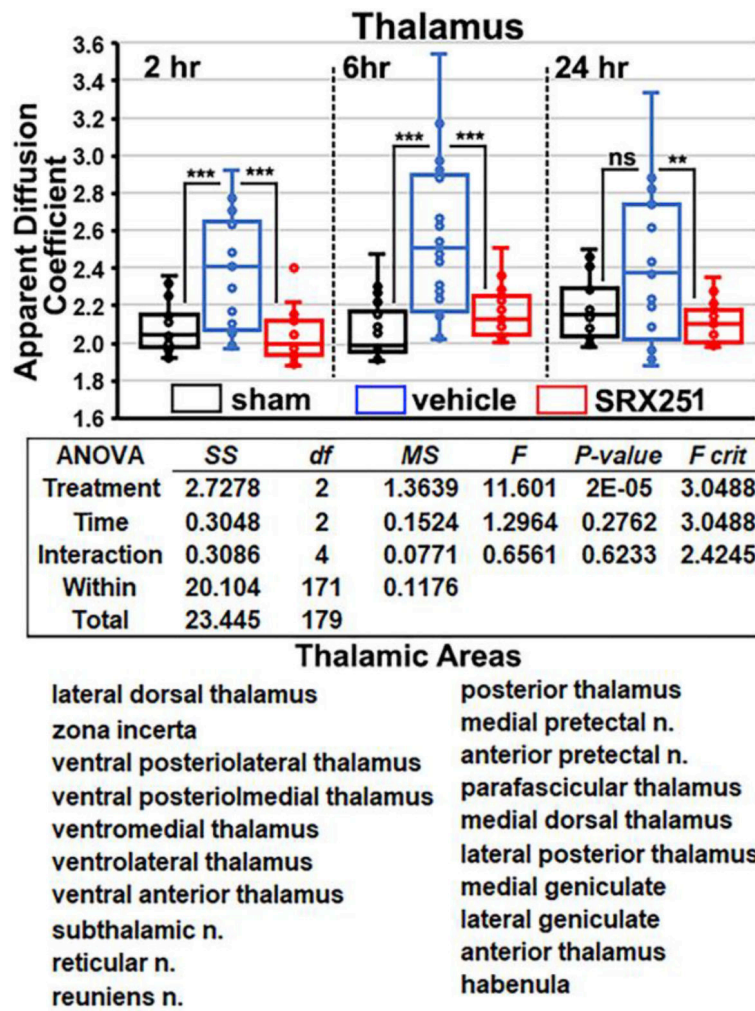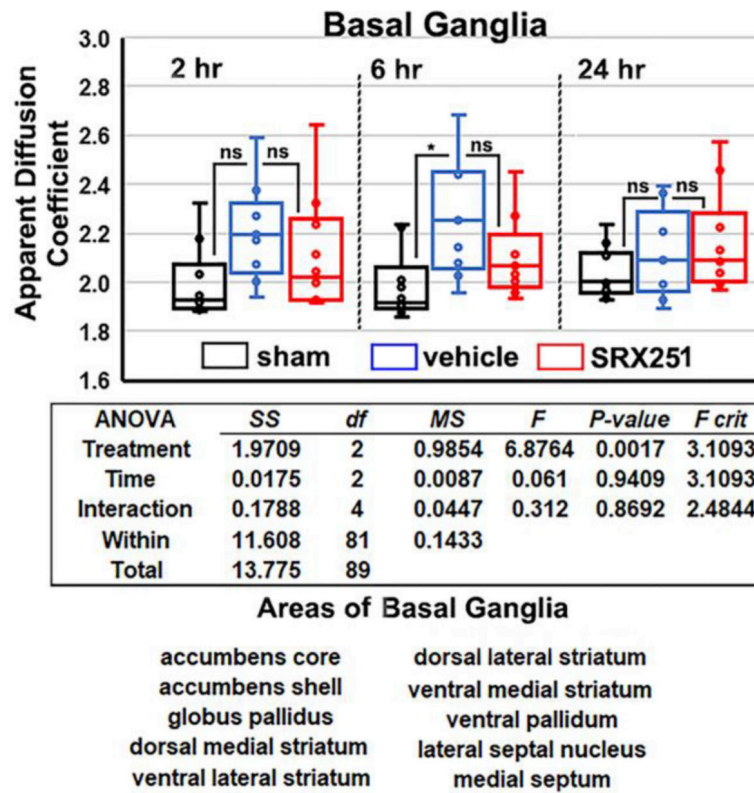|	
1st cerebellar lobule	simple lobule cerebellum
2nd cerebellar lobule	paramedian lobule
3rd cerebellar lobule	paraflocculus cerebellum
4th cerebellar lobule	flocculus cerebellum
5th cerebellar lobule	copula of the pyramis
6th cerebellar lobule	crus 1 of ansiform lobule
7th cerebellar lobule	crus 2 of ansiform lobule
8th cerebellar lobule	medial cerebellar nucleus fastigial
9th cerebellar lobule	lateral cerebellar nucleus
10th cerebellar lobule	interposed nucleus

Figure 2. Mild head impact without evidence of brain damage

Shown are serial coronal sections of T2-weighted anatomical images taken at 2, 6 and 24 hrs post mild head impact. The site of impact is shown by the arrow. The bright area above the skull is fluid from swelling under the skin. These images are from a single rat representative of each experimental treatment.

Thalamus

ANOVA	SS	df	MS	F	P-value	F crit
Treatment	2.7278	2	1.3639	11.601	2E-05	3.0488
Time	0.3048	2	0.1524	1.2964	0.2762	3.0488
Interaction	0.3086	4	0.0771	0.6561	0.6233	2.4245
Within	20.104	171	0.1176			
Total	23.445	179				

Thalamic Areas

lateral dorsal thalamus
zona incerta
ventral posteriolateral thalamus
ventral posteriolmedial thalamus
ventromedial thalamus
ventrolateral thalamus
ventral anterior thalamus
subthalamic n.
reticular n.
reuniens n.

posterior thalamus
medial pretectal n.
anterior pretectal n.
parafascicular thalamus
medial dorsal thalamus
lateral posterior thalamus
medial geniculate
lateral geniculate
anterior thalamus
habenula

Figure 3. Edema in cerebellum

Shown are scatter plots of average ADC values taken from the cerebellum (20 areas shown below) for sham (black), vehicle (blue) and SRX251 (red) treatments 2, 6 and 24 hrs post head injury. ns – not significant, *** $p<0.001$, ** $p<0.01$

Areas of Basal Ganglia

|	
accumbens core	dorsal lateral striatum
accumbens shell	ventral medial striatum
globus pallidus	ventral pallidum
dorsal medial striatum	lateral septal nucleus
ventral lateral striatum	medial septum

Figure 4. Edema in thalamus

Shown are scatter plots of average ADC values taken from thalamus (20 areas shown below) for sham (black), vehicle (blue) and SRX251 (red) treatments 2, 6 and 24 hrs post head injury. ns – not significant, *** p<0.001, ** p<0.01
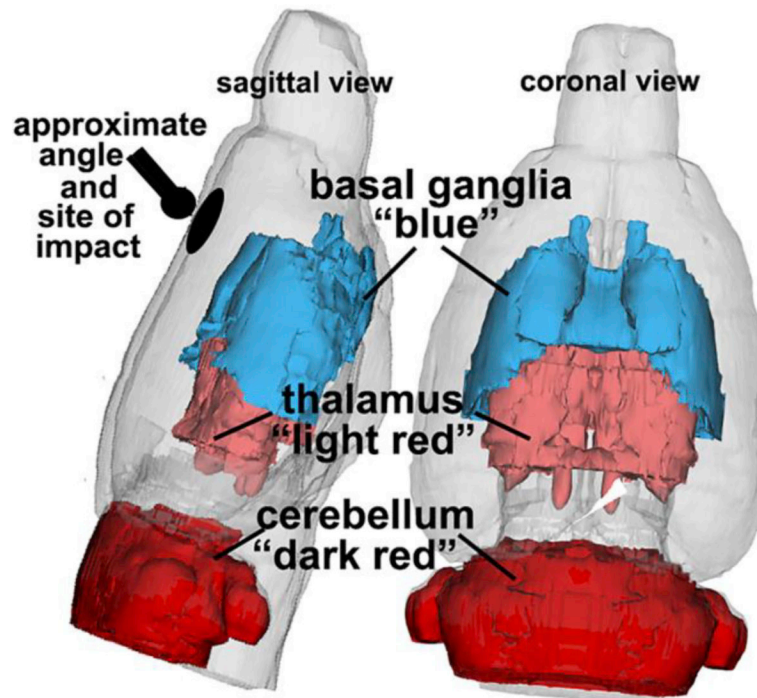
Figure 5. Edema in the basal ganglia

Shown are scatter plots of average ADC values taken from the basal ganglia (10 areas shown below) for sham (black), vehicle (blue) and SRX251 (red) treatments 2, 6 and 24 hrs post head injury. ns – not significant, * p<0.05
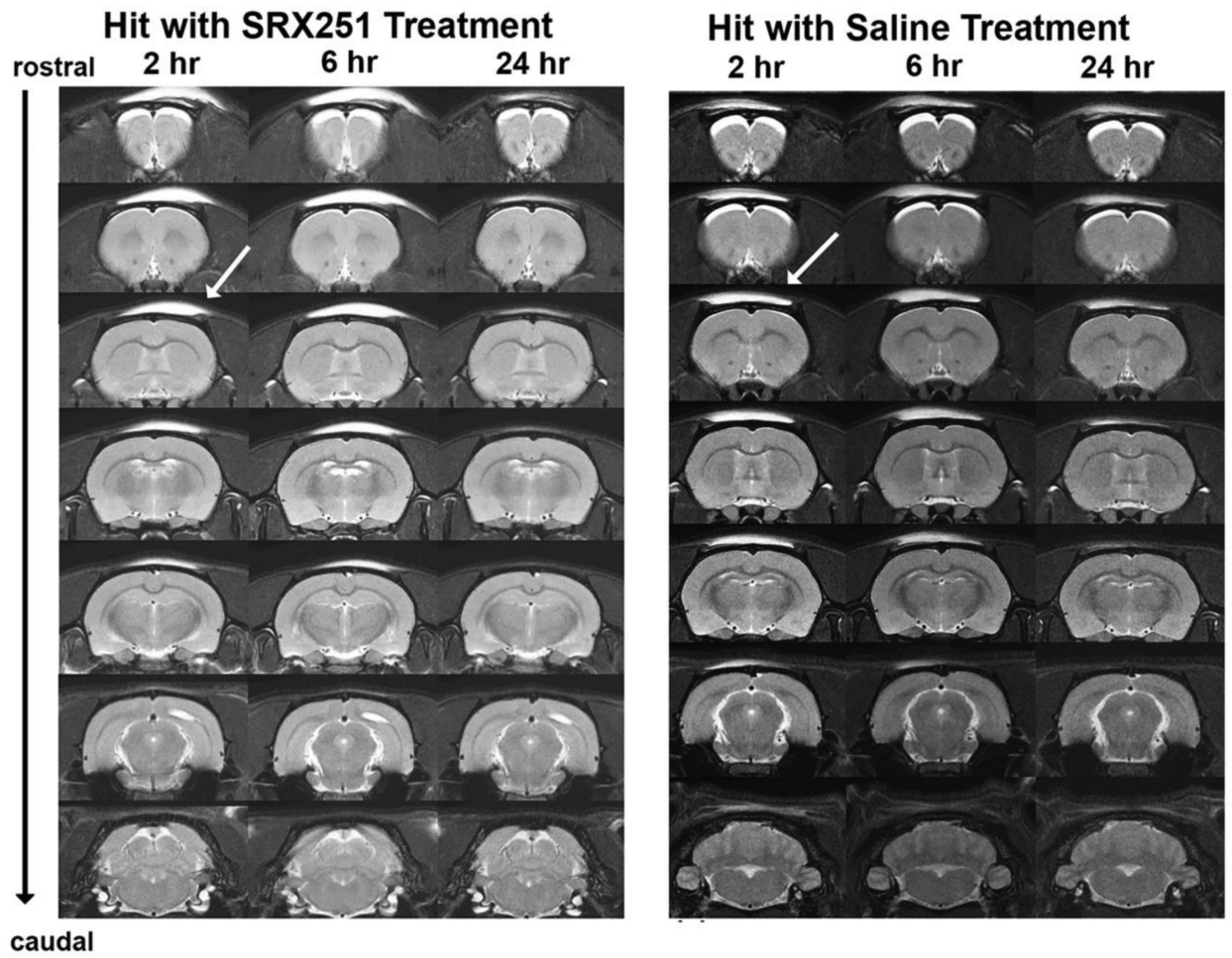
Figure 6. 3D reconstruction of affected brain areas
Shown is a color-coded reconstruction of brain areas showing significant increases in edema following a single mild hit to the forehead (black ball and circle)