Developing an Ovine Model of Impact Traumatic Brain Injury

Charlie C Magarey¹,2, Ryan D Quarrington¹,2,3, Claire F Jones¹,2,4
¹School of Electrical and Mechanical Engineering, The University of Adelaide; ²Adelaide Spinal Research Group, Centre for Orthopaedic & Trauma Research, The University of Adelaide; ³Adelaide Medical School, The University of Adelaide; ⁴Department of Orthopaedics and Trauma, Royal Adelaide Hospital

ABSTRACT

Traumatic brain injury is a leading cause of global death and disability. Clinically relevant large animal models are a vital tool for understanding the biomechanics of injury, providing validation data for computation models, and advancing clinical translation of laboratory findings. It is well established that large angular accelerations of the head can cause TBI, but the effect of head impact on the extent and severity of brain pathology remains unclear. Clinically, most TBIs occur with direct head impact, as opposed to inertial injuries where the head is accelerated without direct impact. There are currently no active large animal models of impact TBI. Sheep may provide a valuable model for studying TBI biomechanics, with relatively large brains that are similar in structure to that of humans. The aim of this project is to develop an ovine model of impact TBI to study the relationships between impact mechanics and brain pathology. An elastic energy impact injury device has been developed to apply scalable head impacts to rapidly rotate the head without causing hard tissue damage. A motion constraint device has been developed to limit the head motion to a single plane of rotation. The apparatus has been tested using deceased animals to assess the controllability of impact velocities, the repeatability of head kinematics, and the dynamic response of the head to impact. Impact velocities are effectively controlled by modulating the elastic energy stored in the impact piston. The resulting head kinematics are somewhat variable, and are influenced by impact location, time dependent post-mortem tissue changes, and specimen head and neck physiology. Model development will continue, and in vivo testing will be conducted to assess the brain pathology following impacts of varying severity.

INTRODUCTION

Traumatic brain injury (TBI) is the leading cause of death globally in people aged under 45 years, and is the most common cause of disability across all age groups (Meaney et al., 2014). In Australia alone, over 20,000 people are hospitalized with a TBI annually (Helps et al., 2008), leading to associated costs of AUD$8.6 billion (Collie et al., 2010). Causes of TBI include sports-related concussions, falls, assaults, road vehicle accidents, and blast and impact loading in conflict zones. Symptoms are dependent on the severity of the injury and the individual’s physiological responses, ranging from nausea and headache to permanent vegetative state and death. Despite substantial global research efforts, brain injury mechanics are not well understood, and no clinical trials of interventions have been successful (Loane & Faden, 2010; Tortella, 2016).
There are three main research streams towards a better understanding of TBI mechanics. Field studies can elucidate where and how TBIs occur clinically, and video tracking (Zimmerman et al., 2022) and wearable sensors (Tierney et al., 2020) have measured head kinematics during the injury event to inform computational and experimental studies. Computational models are used to investigate the macro and microscale mechanics of injury. Experimental models of animals, cadaveric tissue, or human surrogates are used to investigate the kinematics, pathology, and macroscale mechanics of injury. More experimental work, including animal modeling, is needed to properly validate computational models of TBI and improve their clinical relevance (Meaney et al., 2014; Yang & Mao, 2019). To study brain injury mechanics, and validate computational models, it is crucial that animal models closely replicate the mechanism and pathology seen in clinical TBI (Finnie, 2001; Namjoshi et al., 2013; Yang & Mao, 2019).

Most TBIs are closed head injuries (Yang & Mao, 2019), where head rotation is the “predominant mechanism of injury” (Meaney & Smith, 2011). Various animal models have been used to study the physiology and biomechanics of closed head TBI (Johnson et al., 2015; Namjoshi et al., 2013; Vink, 2018), most commonly using rodents (Povlishock, 2016), which have limited clinical relevance due to their small lissencephalic brains (Johnson et al., 2015; Povlishock, 2016; Vink, 2018). Larger animals, such as primates, pigs, and sheep, offer improved clinical relevance as they have relatively large gyrencephalic brains (Cullen et al., 2016; Finnie, 2001; Johnson et al., 2015; S. Murray & N. Mitchell, 2022; Ommaya & Gennarelli, 1974; Vink, 2018). To our knowledge, there is only one current large animal (porcine) model of TBI, which produces injuries through inertial (non-impact) rotation of the head (Cullen et al., 2016). Clinically, the vast majority of TBIs are caused by direct head impact (McLean, 1995; Yang & Mao, 2019), yet there are no currently used large animal models of impact TBI.

The aim of this project is to develop a large animal model of impact TBI in sheep. The model should be repeatable, controllable, and capable of producing a spectrum of injury severities. The TBI should occur without skull fracture, to ensure the model can be used for survival studies with repeated impact events (in the future), and to more closely mimic clinical TBI which most commonly occurs without skull fracture. This manuscript reports the work performed to date on model development and validation against metrics of controllability, repeatability, and anticipated injury production. All testing has been completed using animals humanely killed following unrelated procedures approved by the institutional animal ethics committee.

METHODS

Injury Device

A custom elastic energy impact piston was designed and manufactured (Figure 1A and B). An impact piston was seated within a guiding cylinder via acetal plastic bearing surfaces to provide controlled and consistent impact trajectory. Shock cord (16mm heavy duty, Ibex Marina, Lancashire, UK) was threaded between two mounting plates, one fixed to the guiding cylinder, and the other at the rear of the impact piston. The piston was retracted by a large brake winch (1500 kg, Atlantic Products, South Australia, Australia) via a custom trigger block which locked onto the rear of the piston and was guided by a linear bearing (NAH20, NSK, Tokyo, Japan). As
the piston was retracted by the winch the shock cord was tensioned, storing elastic potential energy. When the desired draw distance was achieved, a custom linear actuator retracted the locking block from the trigger mechanism, releasing the piston. A magnetic linear encoder (±156 μm resolution; LM15, Rotary and Linear Motion Sensors, Komenda, Slovenia) fixed to the piston measured the draw distance and the piston’s displacement and velocity throughout its forward travel. A uniaxial load cell mounted to the piston was used to measure the impact force which was inertially compensated in post-processing (Anderson, 2000). The injury device was mounted to a custom-built stand which was weighted at its base with barbell plates (2 x 10 kg) to reduce the effects of recoil.

Figure 1: A) Schematic of injury device with shock cord extended in the ready to fire position. B) Injury device after firing.
**Planar Motion Constraint**

To standardize head-neck rotation, and to work towards future studies of rotational direction effects, a custom-built planar motion constraint was used to guide rotation of the head in a single plane (Figure 2). The head was fixed to this planar constraint via a bite plate. Modular design allowed the radius of rotation of the head-neck to be altered by moving the vertical beam. The planar constraint was mounted to a custom stand weighted at its base with barbell plates (5 x 10 kg) preventing relative motion of the planar constraint stand and the injury device.

A single-use 3D-printed plastic tray was bolted to a 6 mm thick stainless-steel bite plate (Figure 3A). The tray was filled with polymethyl methacrylate dental acrylic (PMMA; Kulzer, Hanau, Germany) mixed to form a putty consistency (2 parts monomer, 5 parts polymer). The upper teeth were coated with petroleum jelly, the bite plate and tray were inserted into the mouth, and the upper teeth impressed into the PMMA. The plate/tray was then removed from the mouth and the PMMA allowed to cure (Figure 3B). Once set, the construct was returned to the mouth in the same position, and fixed to the upper jaw and snout with cable ties and/or wire (Figure 3C). The mouth, below the plate, was packed tightly with gauze and the mandible held firmly closed with cable ties and/or wire (Figure 3D).
Figure 3: A) Schematic of bite plate (green) and 3D printed tray (blue). B) 3D printed tray with PMMA with teeth imprint. C) Bite plate fixed to upper jaw. D) Mouth packed tightly with gauze and clamped around bite plate.

“Helmet”
A custom steel “helmet” was designed to conform to the contour of the skull and provide an impact surface that is approximately perpendicular to the radius of rotation of the head and planar constraint (Figure 4A-B). Butadiene rubber (4 mm thick) was adhered to the impact surface of the helmet to prevent metal-on-metal contact between the steel piston head and helmet, which would otherwise transmit high-frequency vibration to sensor array mounted on the head. Neoprene rubber (8 mm thick) was placed between the helmet and the head surface to increase contact area and distribute loads. The helmet was fixed to the animal’s head with Velcro straps (Figure 4C).
Sensor Array

A custom sensor array was used to measure the kinematics of the head during injury. Linear kinematics were measured using four triaxial accelerometers (35B-2, Endevco, North Carolina, USA) in a 3-2-2-2 configuration, and angular kinematics determined using these accelerometers and three orthogonal uniaxial angular rate sensors (ARS PRO-18K, DTS, California, USA). To fix this array to the head, first the scalp was resected from the cranium, exposing the bregma and the surrounding bone (Figure 5A). The pericranium was removed from a section of bone (approximately 50 x 50 mm) posterior to the bregma. Four pilot holes (2 mm diameter) were created with a hand drill, penetrating only the outer cortical layer. Self-tapping wood screws (16 mm long, 4G) were seated in the pilot holes without penetrating the inner cortex and protruding from the bone outer surface. Wire was wrapped around the protruding screw shafts (Figure 5B) and then an approximately 60 mm (anterior-posterior) x 50 mm (lateral) x 30 mm (superior) mantle of PMMA (2 parts monomer, 5 parts polymer) was built up around the wire and screws (Figure 5C). Prior to complete cure, an interface plate was fixed to the PMMA mantle with four wood screws (16 mm 4G), and the array was fixed to this interface plate via threaded holes (Figure 5D). The array was aligned with the head of the animal such that rotational axis of the array closely approximated the rotational axis of the head.
Data Collection and Processing

Sensor signals were collected using a data acquisition system (cDAQ-9178; 4 x NI-9232, accelerometers; NI-9215, ARS; NI-9237, load cell; National Instruments), at 50 kHz via custom LabView code, and all signals were synchronized to a single internal clock of the cDAQ. Two high speed cameras (Phantom VEO1010, Vision Research, New Jersey, USA) were used to record injury tests at 21,000 fps for qualitative analysis. Post-processing was completed in MATLAB (2021b, Mathworks, Massachusetts, USA) and data filtered with a second order low pass Butterworth filter with cut-off frequencies determined through Fast Fourier Transform analysis in accordance with SAE J211. Kinematics data was expressed in the sensor array coordinate system, where rotational axis closely approximated that of the head due to array alignment.

Shock Cord Tension Testing

Prior to testing, piston draw distance was calibrated to shock cord tension, measured by an additional load six-axis load cell (K6D110 ±20 kN, ME, Hennigsdorf, Germany) attached between the winch and the trigger release mechanism. The shock cord tension versus piston draw distance relationship was defined and used to select appropriate draw distances in subsequent impact testing.
Deceased Animal Testing Procedure

Testing to date has been completed using full deceased animals, isolated heads, and isolated head-necks with the second thoracic vertebrae fixed to the planar motion constraint stand. All deceased tissue has been scavenged from animals humanely killed at the completion of unrelated procedures approved by the institutional animal ethics committee. The recently deceased (~10 minutes) animal was placed prone on a surgical table, the sensor array, helmet, and bite plate were fixed to the head, as described above. The animal was placed in left lateral recumbency with the body on the surgical table, and the head fixed to the planar constraint via the bite plate, such that the head and neck are aligned with the thoracic spine when the planar constraint is in its central position. The planar constraint was rotated counterclockwise approximately 45 degrees, putting the head into extension (Figure 6). The injury device was positioned such that the impact surface of the helmet was aligned with the front end of the injury device cylinder. The head was initially placed in extension at the time of impact, this allowed the head to rotate sagittally through the neutral posture and then into flexion. The piston was retracted via the winch to the desired draw distance, then the trigger was actuated, and the head impacted. Impact tests were repeated until the onset of rigor mortis had a clear effect on the neck stiffness.

Figure 6: Deceased animal fixed to planar motion constraint and positioned into neck flexion prior to an impact injury trial.

Tests were performed to assess the controllability and repeatability of the injury apparatus, and to investigate the impact dynamics of the head. Controllability was assessed to determine if head rotational kinematics, piston impact load and velocity, and shock cord tension can be controlled by controlling the draw distance of the shock cord. Inter- and intra-specimen repeatability was assessed to determine if tests of comparable impact piston velocity would result in comparable head rotational kinematics. Rotational kinematics of the head and the planar
constraint were compared to investigate how the head responds to impact, and how this impact energy is transferred through the head to the planar constraint. Data presented in this manuscript is from head, head-neck, and full animal testing of the latest injury apparatus iteration (Table 1).

<table>
<thead>
<tr>
<th>Specimen Name</th>
<th>Specimen Type</th>
<th>Testing Performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH1</td>
<td>Isolated head</td>
<td>6 impacts at various severity</td>
</tr>
<tr>
<td>DA1</td>
<td>Deceased animal</td>
<td>3 impacts at constant severity</td>
</tr>
<tr>
<td>DA2</td>
<td>Deceased animal</td>
<td>3 impacts at constant severity</td>
</tr>
<tr>
<td>DHN1</td>
<td>Isolated head and neck</td>
<td>3 impacts at constant severity</td>
</tr>
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### RESULTS

**Controllability**

The relationship between the piston draw distance and the tension of the shock cord was non-linear to approximately 28 mm draw, then approximately linear from 28 mm to 138 mm draw (Figure 7). This confirmed that the tension of the shock cord, and therefore the energy delivery to the head, could be controlled by varying the draw distance of the piston.

![Figure 7: Mean and trial piston draw distance versus shock cord tension. Initial length of untensioned shock cord = 195mm.](image)

Six head impact tests were performed on specimen DH1. Piston impact velocity, impact force, and peak sagittal rotational velocity of the head all increased with increasing draw distance (Table 2). The relationships between piston draw distance and piston impact velocity, and between piston draw distance and peak head sagittal rotational velocity, were approximately linear (Figure 8).
Table 2: Isolated head impact results for varying piston draw distances

<table>
<thead>
<tr>
<th>Draw Distance (mm)</th>
<th>Shock Cord Tension (kN)</th>
<th>Impact Velocity (m/s)</th>
<th>Peak Impact Force (kN)</th>
<th>Peak Sagittal Rotational Velocity of Head (rad/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.28</td>
<td>2.23</td>
<td>4.13</td>
<td>5.16</td>
<td>25.1</td>
</tr>
<tr>
<td>35.94</td>
<td>2.30</td>
<td>4.52</td>
<td>6.05</td>
<td>31.7</td>
</tr>
<tr>
<td>42.34</td>
<td>2.45</td>
<td>4.75</td>
<td>8.04</td>
<td>41.3</td>
</tr>
<tr>
<td>76.87</td>
<td>3.14</td>
<td>8.95</td>
<td>14.0</td>
<td>127</td>
</tr>
<tr>
<td>79.37</td>
<td>3.19</td>
<td>9.17</td>
<td>13.5</td>
<td>156</td>
</tr>
<tr>
<td>114.5</td>
<td>3.93</td>
<td>13.6</td>
<td>14.7</td>
<td>214</td>
</tr>
</tbody>
</table>

Figure 8: A) Relationship between piston draw distance and piston impact velocity. B) Relationship between piston draw distance and peak head sagittal rotational velocity (SRV).

Repeatability

Three impact tests were performed on each of two deceased animals (DA1 and DA2, Figure 9A-D). The helmet and sensor array positions were changed between these two animals; DA1 shows outdated positioning (Figure 9C), and DA2 matches the positioning described in the methods section (Figure 9D). Intra-specimen trend of the head sagittal rotational velocity appeared comparable, while peak values showed some variation. Inter-specimen trends appeared less comparable, with an initial positive peak in sagittal rotational velocity seen in DA1 tests (Figure 9A), but not in the tests of DA2 (Figure 9B).
Figure 9: A-B) Head sagittal rotational velocity (SRV) from two deceased animals, all tests were at approximately the same piston draw distance (75.7 ± 1.2 mm), time=0ms corresponds to contact onset. C) Deceased animal 1 test setup. D) Deceased animal 2 test setup.

Peak absolute sagittal head rotational velocity showed high coefficient of variation (CoV) (~25%) for both inter- and intra-specimen comparisons (Table 3). Time-to-peak value showed low intra-specimen CoV (≤ 5%), and high inter-specimen CoV (34%).

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Table 3: Inter and intra specimen mean, standard deviation (SD) and coefficient of variance (CoV) for the peak absolute sagittal rotational velocity (SRV) and time to peak value for two deceased animals.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>CoV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deceased Animal 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak abs SRV (rad/s)</td>
<td>51.25</td>
<td>11.76</td>
<td>23%</td>
</tr>
<tr>
<td>Time to abs peak (ms)</td>
<td>2.29</td>
<td>0.08</td>
<td>4%</td>
</tr>
<tr>
<td><strong>Deceased Animal 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak abs SRV (rad/s)</td>
<td>63.51</td>
<td>15.25</td>
<td>24%</td>
</tr>
<tr>
<td>Time to abs peak (ms)</td>
<td>1.21</td>
<td>0.06</td>
<td>5%</td>
</tr>
<tr>
<td><strong>All Tests</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak abs SRV (rad/s)</td>
<td>57.38</td>
<td>15.73</td>
<td>27%</td>
</tr>
<tr>
<td>Time to abs peak (ms)</td>
<td>1.75</td>
<td>0.6</td>
<td>34%</td>
</tr>
</tbody>
</table>

Impact Dynamics

Peak sagittal rotational velocity of the head in an isolated head and neck test was 54.4 rad/s, while peak rotational velocity of the planar motion constraint was 5.7 rad/s (Figure 10A). Rotational kinematics of the head and the planar motion constraint differed during and immediately following the impact event, and then began to converge approximately 50 ms post impact (Figure 10A-B).

Figure 10: Rotational velocity (A), and rotation (B) of the planar motion constraint apparatus, and of the head in the sagittal plane in isolated head and neck testing at piston draw distance of 77 mm.

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DISCUSSION

The aim of this project was to develop an ovine model of impact induced traumatic brain injury. The work to date has included iterative improvements to the injury apparatus via pilot testing of deceased animals, isolated heads, and isolated head-neck specimens. Test metrics included controllability, repeatability, and whether resultant head kinematics are expected to produce TBI through comparison to other animal models and human injuries. Results presented in this manuscript are of the tests performed with the most recent configuration of the model. Most of this testing has been with the impact piston drawn approximately 75 mm (40% of the maximum injury device draw distance), as this allowed a single specimen to be tested multiple times without hard tissue damage, while still producing seemingly severe impacts.

Elastic shock cord provided a highly controllable means of energy delivery to the head. The tension in the shock cord could be controlled by varying the initial draw distance of the piston (Figure 7). Piston impact velocity (Figure 8A), impact force, and resultant head rotational velocity (Figure 8B) all increased with increasing draw distance. Relationships between draw distance and piston velocity, and between draw distance and head rotational velocity, appeared approximately linear (Figure 8A-B), although more tests are required to accurately quantify these relationships. These relationships will allow for targeting of specific injury severity or resultant head kinematics by setting the appropriate piston draw distance.

Peak head sagittal rotational velocity showed relatively high CoV of 24% and 27% respectively (Table 3). A number of limitations in the current apparatus and testing protocol likely contributed to this variability. Firstly, a consistent piston draw distance was targeted for all tests, but due to the discrete locking intervals of the winch there was some variation between trials (SD 1.2 mm). In future testing, a hard stop will be fitted to the linear bearing that guides the piston retraction, to ensure draw distance is consistent between trials. A consistent intra-specimen impact location was also targeted, with the helmet fitting checked and adjusted between trials if necessary, but small changes in helmet position may still have affected the energy transfer to the head. Testing was ceased at the first palpable signs of rigor mortis, but time dependent post mortem changes to the neck musculature may have affected test results. The onset of rigor also limited the number of tests that could be repeated on a single specimen, and this low number of tests is likely inflating the measure of variation.

Head sagittal rotation versus time (Figure 9A-B) showed good intra-specimen repeatability. This was supported by the low variation (CoV ≤ 5%) in the time-to-peak value for both specimens (Table 3). However, there were clear differences in the head response between the two specimens (Figure 9A-B), which also showed a large variation (CoV 34%) in time-to-peak value. DA1 (Figure 9A) showed an initial positive oscillatory region before reaching the absolute peak, while DA2 (Figure 9B) showed an initial negative peak, followed by a smaller positive peak. These initial differences between specimens converged over time as the head transitioned from the dynamic impact response to the gross motion guided by the planar motion constraint. While the impact location was controlled for all tests in the same animal, it was changed between animals to support an improved mounting point of the sensor array directly to the cranium (Figure 9C-D). We hypothesize that these changes in impact location, impact trajectory, and array position contributed to the differing trend in head kinematics between these animals. This will be investigated with

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future testing of deceased animals, or isolated head and necks, with consistent impact and sensor locations.

There was a clear difference in the rotational kinematics of the head and the planar motion constraint within the first ~50 ms post impact, followed by convergence for the remainder of the motion (Figure 10A-B). The head underwent rapid rotation and oscillation during, and immediately following, impact, while the planar motion constraint showed a relatively gradual acceleration to a peak value which remained approximately constant for the remainder of the motion (Figure 10A). Qualitative analysis of high-speed video footage showed the initial peak in the sagittal rotation of the head was caused by rotation of the cranium due to local deformation of the skull at the impact site (Figure 11A and B). The following negative peak occurred as the dense cranial region of the head pivoted about the relatively deformable snout region (Figure 11A and C). As more energy was transferred to the planar motion constraint, the head rotation trended positive and began to converge with the rotation of the planar motion constraint (Figure 11A and D). Kinematics of the head in the initial period of motion (~50 ms) were driven primarily by the head response to impact, with the planar motion constraint limiting motion to the sagittal plane, but not to the targeted direction.

The susceptibility of a brain to injury is proportional to its mass, with larger brains being exponentially more susceptible to injury at the same acceleration (Browne et al., 2011; Holbourn, 1943; Ommaya et al., 1967). The mass of the adult sheep brain is approximately 130-140 g (S. J. Murray & N. L. Mitchell, 2022), comparable to that of the adolescent swine (80 – 150 g) used in another TBI model (Cullen et al., 2016). In this swine model, injury was predicted to occur at 95-120 rad/s (based on scaling to human injury) and shown experimentally to produce coma of several hours duration at 110 rad/s (Cullen et al., 2016). These rotational velocities (95-120 rad/s) will be used as the target value for injury in our sheep model. Mean peak rotational velocity of the head in deceased animal testing was 57 rad/s at just 40% of the maximum injury device output/draw distance. Due to the steep linear increase of head kinematics with increasing piston draw distance (Figure 8B), this model is expected to produce TBI-inducing head rotations at higher piston draw than used thus far. Differences in injury mechanism, loading rate and skull deformation limit the reliability of this comparison.

This sheep model of impact TBI is intended to produce injury across a spectrum of severities. Preliminary assessment showed the apparatus to be controllable, with head kinematics and impact severity being controlled by controlling the draw distance of the impact piston. Limitations related to the injury apparatus’ repeatability were identified, and will be addressed as model development continues. Further deceased animal testing is required to predict the spectrum of injury severity achievable with this new model, although preliminary results indicate that injurious head kinematics will be achievable. Further development and quantification of this model will lead to in vivo testing, for histological analysis of brain pathology across impact severities.
CONCLUSIONS

Development of an elastic potential energy impact model of TBI in sheep is underway. Preliminary results of deceased animal testing have shown that impact severities and peak head rotational velocities can be controlled by modulating the draw distance of the impact piston. Limitations related to the injury apparatus’ repeatability have been identified and will be addressed in further model development. Investigation of the impact dynamics of the head highlight the difficulty in controlling skull impact effects, which is a tradeoff for the improved clinical relevance of an impact injury model. Further development of this model will use in vivo testing to assess the...
brain pathology produced at various injury severities. Future studies using this model will include investigating the deformation of the brain during injury, and the effects of multiple concussive injuries on subsequent brain pathology.

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