BIOMECHANICS OF A SHEEP MODEL OF AXONAL INJURY

RWG Anderson^a, CJ Brown^b, G Scott^c, PC Blumbergs^c, JW Finnie^c, AJ McLean^a, NR Jones^b

^a NHMRC Road Accident Research Unit, University of Adelaide, Australia, ^b Dept. Surgery (Neurosurgery), University of Adelaide, and ^c Div. of Tissue Pathology, Institute of Medical and Veterinary Science, Adelaide

ABSTRACT

The aim of this project was to investigate the feasibility of using an animal model of axonal injury to study the biomechanics of the injury. The model utilises anaesthetised sheep that are mechanically ventilated and stabilised before being subjected to a single lateral impact from a captive bolt gun. The impact force was measured using a load cell mounted in the striker, and the resulting head acceleration was measured by means of a 9-accelerometer array which was rigidly mounted to the head of the sheep. Head kinematics were transformed to anatomical coordinates using stereo-radiography. High speed cine film (1000 fps) was used for the visualisation of gross head motion. After impact, each animal was allowed to survive for a predetermined period during which anaesthesia was maintained. A complement of physiological monitors was used to measure the physiological state of the animal at all times during the experiment. In one experiment, hypoxia was induced after the physical insult. After the survival period, the animal was sacrificed and the brain removed for histological processing. The brain was sectioned, processed and examined for axonal injury using the presence of amyloid precursor protein (APP) as an indicator of injury. The distribution of axonal injury in serial sections of the brain was mapped and quantified. Five experiments, displaying a range of injury responses, are reported on in this paper. In the future, the model will be used to study the biomechanics of axonal injury.

All experiments conform to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

AMONG PERSONS SUSTAINING A HEAD INJURY, axonal injury is a major cause of mortality and morbidity (Gennarelli, 1984). There is evidence that it occurs across the spectrum of head injury severity (Blumbergs et al., 1994). Yet it remains unclear to what extent different mechanical parameters contribute to the incidence of axonal injury, and how post impact physiological changes relate to the eventual severity of axonal injury.

There have been many biological studies which have correlated the incidence of 'diffuse type' brain injuries (which includes axonal injury) with loading parameters. These types of studies have included isolated tissue studies, studies of single tracts of axons, direct percussion of the brains of small animals, and inertial loading of the head of larger animals. Animal models have the advantage that they are more likely to be better physiological representations of the human brain that isolated axon preparations. Isolated tissue studies are usually more simple to model biomechanically, however.

Several investigators have used utilised single axons or single bundles of axons to study the response of the axon to loading. These studies have shown that direct mechanical loading of axons can lead to physiological changes in the cell such as rises in intracellular calcium (Saatman and Thibault, 1991) and increased rate of glucose metabolism (Gennarelli et al., 1989). Such models have also produced clear evidence that applied loads that are less than that required to cause primary axotomy may result in marked histological changes (Gennarelli et al., 1989). If brain tissue deformation can produce marked physiological as well as histological

changes, it is important that animal models of axonal injury include monitoring and control of the animal's pre- and post-impact physiology. Any physiological effects on the nature and extent of the resulting injury may then be measured and normalised.

Percussion and direct cortical impact models are animal models in which the cortex of the brain of the living animal is subjected to a pressure pulse, usually through a craniectomy. Insofar as they have been used for biomechanical analyses, these types of models have generally been used as part of an effort to study the injury response of brain tissue subjected to deformation (Ueno et al., 1991; Ueno et al., 1996; Meaney et al., 1994a).

Early studies using large animal models examined the relationship between the qualitative characteristics of inertial loading of the head, and the resulting 'diffuse' injury (Hirsch and Ommaya, 1970; Ommaya and Hirsch, 1971) or axonal injury (Gennarelli et al., 1982; Gennarelli et al., 1987). In some of these studies, axonal injury was not observed directly, but was inferred from other observations such as the duration of traumatic coma. More recent animal studies have examined the relationship between the kinematic parameters, the tissue level strains and the incidence of axonal injury (Margulies et al., 1990; Mendis, 1992; Meaney et al., 1993; Meaney et al., 1994b; Meaney et al., 1996; Miller et al., 1996). Studies designed to examine brain tissue deformation have often used some form of numerical and/or physical modelling to estimate levels of strain. Some attempts have been made to compare the estimates of tissue strain and the resulting injury in the animal (Ueno et al., 1996; Meaney et al., 1994b). Early animal models used primates to maintain some anatomical fidelity with humans, and scaling laws were derived in an attempt to relate the results to human beings (see for example Ommaya et al., 1966; Ljung, 1980). However, advances in numerical modelling have meant that estimates of injury tolerance can now be made at the tissue level and have allowed other species to be used.

Several recent investigations have shown promising results in correlating the incidence of axonal injury with kinematic data and the estimated levels of strain in the brains of animals subjected to rapid dynamic loading. Meaney (1994b) used a physical model simulation of experiments in which a pig's head was subjected to a coronal plane angular acceleration. They found that by incorporating the direction of axon bundles in the pig brain, to formulate what they described as a level of 'oriented strain', they were able to predict the incidence of injury in the animal to a reasonable degree. Analysing the same animal experiments, (Miller et al., 1996) quantified the extent and the severity of axonal injury. They were able to correlate the proportion of the tissue injured in certain regions of the brain with the peak angular acceleration and the maximum change in angular velocity.

New animal models of axonal injury are still required, even though the study of diffuse type brain injuries in living animals has been taking place for more than 30 years. Advances in kinematic measurement techniques, numerical modelling techniques, and the use of previously unavailable histological methods, have greatly enhanced the ability of researchers to investigate the mechanisms of axonal injury.

There has been no attempt thus far to examine the relationship between dynamic parameters and the severity and distribution of axonal injury in a large animal subjected to a concentrated impact loading to the head. In a study of real life traffic accidents, brain injury without some form of head contact was not seen (McLean, 1996). Any added effects of post impact physiology on the severity and distribution of axonal injury has also not been satisfactorily addressed in previous animal models. The development of the animal model presented in this paper was prompted, in part, by the paucity of information about the influence of these factors on the resulting injury.

AIM

The aim of this study is to examine the feasibility of developing a controlled and reproducible model of axonal injury due to impact loading of the head. The model will be used to study the biomechanics of axonal injury and the effect of post impact hypoxia on the extent and nature of the injury. This paper reports on the biomechanical development of the model, and presents the results of five experiments. In the last experiment, an attempt was made to impose a period of hypoxia upon the sheep after the impact.

EXPERIMENTAL PROTOCOL

Each experiment took place over three days; the first day was used to prepare the animal by implanting a saggital sinus Doppler crystal for blood flow measurements. The head impact part of the experiment was performed after two days to allow the crystal to become adherent to the dural surface.

DAY ONE PROTOCOL - A two year old merino ewe was induced into anaesthesia with thiopental (15 mg/kg), and intubated. Anaesthesia was then maintained with 2 per cent isofluorane in 50 per cent oxygen and 50 per cent nitrogen, delivered via an endotracheal tube and controlled mechanical ventilation.

A craniotomy was performed and a Doppler crystal to measure blood flow was positioned over the superior saggital sinus. The craniotomy was close and the isofluorane was withdrawn. Prior to the end of the procedure 1.5 ml of Finadyne was given for analgesia.

DAY THREE PROTOCOL - Anaesthesia was induced with diazepam (1 mg/kg), and ketamine, (4 mg/kg). The animal was then intubated. The animal was mechanically ventilated under isofluorane (2 per cent), until a ketamine intravenous line was established. The animal was ventilated with 100 per cent oxygen (3 l/min), until blood gases could be verified. Anaesthesia was maintained by an intravenous infusion of a ketamine/saline solution (15 mg/kg/hr) and isofluorane (1 per cent). Blood gas samples were taken at 10 minute intervals for the duration of the experiment. Ventilation was adjusted to maintain a target of 40 mmHg PaCO₂ and a target of 110 \pm 10 mmHg PaO₂ (core temperature corrected values). Physiological parameters were digitally recorded throughout the experiment. The parameters monitored were cerebral blood flow (CBF), central venous pressure (CVP), arterial blood pressure (ABP), sagittal plane electrocardiogram (ECG), intracranial pressure (ICP), core temperature and fluid balance.

The accelerometer array was attached rigidly to the skull of the animal as described in the next section. The muzzle of a captive bolt gun was positioned so that the striker would predominantly contact the parietal bone and to a lesser extent the temporal bone (see Figure 1).



Figure 1 Impact location

Once all physiological parameters were stabilised within tolerance limits, a 30 minute baseline period was commenced.

At the end of the baseline period an impact sequence was initiated. The captive bolt gun was fired and the resulting motion of the head was recorded on high speed film (HyCam, 1000 fps). All biomechanical measurements were captured via an A/D card installed in a digital computer. The animal was then repositioned, and the endotracheal tube reconnected immediately. Anaesthetic was continued for a further 2 to 4 hours and all physiological parameters were continuously monitored during this period.

In the last experiment, a period of hypoxia was imposed. The oxygen supply was altered so that the animal's PaO_2 was reduced to 15 mmHg for 15 minutes. During this period, blood gases were measured every two and a half minutes. After 15 minutes the oxygen supply and PaO_2 were returned to normal target values.

At the end of the survival period each animal was sacrificed by perfusion fixation with 4 per cent

para-formaldehyde. The skull was opened using a craniotome and the brain was removed. The brain was then placed in 4 per cent para-formaldehyde for two weeks.

The head was removed from the animal and the skull stripped of soft tissue using an enzyme solution. The skull was examined for fracture and stereo-radiographed in order to determine parameters for the subsequent biomechanical analysis.

BIOMECHANICAL MEASUREMENTS

CAPTIVE BOLT GUN - The impacts were delivered to the sheep's head using a modified Schermer MKL captive bolt gun which can be mounted on a rigid frame. The modified captive bolt gun assembly is shown in Figure 2. The striker is powered by the expanding gases generated when a cartridge is detonated in the cartridge chamber. The striker is guided before striking the animal.

The gun was modified so that the dynamics of the striker could be measured; the impact force by a load cell mounted in the striker, and the relative striker velocity by an array of Hall-effect switches mounted in the muzzle. Four different charge strengths are available ("11", "13", "17" and "21"), allowing a range of striker velocities. Two versions of the striker were built and tested; the first is shown in the lower half of Figure 2 and the second, shown mounted in the captive bolt gun. The main difference between these two strikers was their mass. Both strikers present a domed surface of about 50 mm diameter.

In the initial experiments reported on here, the captive bolt gun was mounted on a rigid frame to minimise recoil velocity during firing. In later experiments the gun was hand held.



Figure 2 Section view of the modified captive bolt gun assembly. (1) Displacement sensing muzzle, (2) Striker, (3) Force transducer, (4) Barrel, (5) Bolt, (6) Cartridge, (7) Trigger

ACCELERATION MEASUREMENTS - An array of nine accelerometers may be so arranged so that the calculation of angular acceleration about each of the body-fixed axes at each time point is independent of any previous time points. This nine accelerometer array formation has been discussed extensively elsewhere (Padgaonkar et al., 1975; Mital and King, 1979; Alem and Holstein, 1977; Melvin and Shee, 1989; Boghani et al., 1989). The equations for the angular acceleration of such an array are:

$$\dot{\omega}_{x} = (\ddot{z}_{1} - \ddot{z}_{0}) / 2\rho_{1} - (\ddot{y}_{3} - \ddot{y}_{0}) / 2\rho_{3}$$

$$\dot{\omega}_{y} = (\ddot{x}_{3} - \ddot{x}_{0}) / 2\rho_{3} - (\ddot{z}_{2} - \ddot{z}_{0}) / 2\rho_{2}$$

$$\dot{\omega}_{z} = (\ddot{y}_{2} - \ddot{y}_{0}) / 2\rho_{2} - (\ddot{x}_{1} - \ddot{x}_{0}) / 2\rho_{1}$$
(1)

(Padgaonkar et al., 1975), where the symbol ρ denotes the array arm lengths, and the other variables denote acceleration measured at individual accelerometers within the array (see Figure 3). An accelerometer array that utilises the system of the above equations to measure rigid body motion was designed and built for this study (see Figure 3). The array base was milled from a solid block of aluminium, and nine piezo-electric accelerometers (Brüel & Kjær

type 4901) were mounted on the base to measure the required accelerations. These accelerometers operate in the range 0.1 Hz-16 kHz making them suitable for the measurement of the short impulsive accelerations experienced by the animals in this study.

COMPENSATION FOR NON-IDEAL ACCELEROMETER ARRAY CHARACTERISTICS - In the array configuration presented above, the seismic centres of the accelerometers are offset from the principal axes of the array. This is associated with a measurement error due to the centripetal accelerations experienced by accelerometers that have their sensitive axis perpendicular to, and displaced from the axis of rotation (DiMasi, 1995). This, in addition to the cross axis sensitivity, bias and placement errors of the accelerometers, is likely to add spurious components to the measurement of the actual acceleration of the array. The techniques for the calibration of this type of array have been discussed extensively by others (Boghani et al., 1989; DiMasi, 1995; Plank et al., 1989). For the accelerometer array presented above, many of the required compensation coefficients theoretically reduce to zero because each accelerometer's sensitive axis lies in one of the planes formed by the axes of the array. This means that there is effectively no misalignment of accelerometers insofar as the calculation of angular acceleration is concerned. Theoretically, the only compensation required is that for the linear acceleration measured at the origin of the array.



Figure 3 Accelerometer array to measure head kinematics

ACCELEROMETER ARRAY ATTACHMENT - A mounting system for the accelerometer array was manufactured such that the array could be rigidly attached to the sheep's head. The design allowed the plate to be reattached after the experiment when the skull of the animal had been cleaned, so that the relative position of the array with respect to the sheep's bony anatomy could be determined.

The mounting plate consists of a 3 mm plate of aluminium which extends over the cornual processes of the sheep skull. The plate extends forward and down, through a bend of 45°, over the frontal bone (see Figures 1 and 4). Two dowels are incorporated into the plate to facilitate accurate alignment of the accelerometer array with the plate.

The mounting plate underwent a series of modifications throughout the study, in an attempt to make the array to skull attachment as stiff as possible. These modifications included the removal of spacers, the inclusion of more screws and bone reinforcing.

TRANSFORMATION OF MEASURED ACCELERATIONS TO AN ANATOMICAL VECTOR BASE - The calculated accelerations were measured with respect to the array coordinate system. Anatomical coordinate systems were defined for each animal so that the experimental results were comparable, and so that they will be able to be more readily applied to a finite element model in the future.

To express the acceleration of the head in terms of a consistent anatomical coordinate system, the vector transformation between the array coordinate system and the anatomical coordinate

system had to be determined for each experiment. This was achieved by attaching radio opaque markers and taking two orthogonal x-ray images of the skull and array base plate, to define the relative orientation of the array and the skull. In summary, the technique to do this involved:

- the definition of an anatomical coordinate system by attaching radio opaque markers to conveniently chosen anatomical landmarks on the skull,
- the definition of the instrumentation coordinate system by the use of radio opaque markers,
- the use of stereo-radiography to measure the location of the above markers in the laboratory space,
- from these measurements, the derivation of a mathematical transformation that allows a vector defined in the instrumentation base to be defined in the anatomical base.

This transformation was then used to estimate the acceleration of the anatomical base from the array measurements. Similar techniques are described by Becker (1977) and Nusholtz et al., (1979).



Figure 4 Instrumentation and anatomical coordinate systems.

This procedure was performed *in vivo* before the impact in the first two experiments. For the subsequent experiments, the procedure was performed *in vitro*, after the skull was removed and cleaned. The advantage of performing the procedure before the impact is that the plate does not have to be reattached after the experiment, minimising any realignment error. The disadvantage is that accurate marker placement on the anatomy *in vivo* is more difficult than placement *in vitro*. Another advantage of performing the procedure *in vitro* is that many more marker sites are available, with the whole bony anatomy exposed. The imaging is also more accurate, with less chance of the subject moving between exposures. For these reasons the *in vitro* procedure is preferable.

DATA ACQUISITION AND POST PROCESSING - All kinematic data was captured using an A/D expansion card and a simple acquisition program on a PC. These signals were then post-processed to calculate the force and acceleration histories.

NEUROPATHOLOGY

To assess the degree of axonal damage, a survey of axonal lesions was undertaken in each case and the distribution of axonal lesions, as defined by the presence of axonal amyloid precursor protein (APP), was mapped.

After the brain of the animal was completely fixed, it was cut into at least sixteen 5 mm coronal slices. Each slice was embedded in paraffin wax and five micron sections were cut and prepared for examination using standard H & E staining & anti-APP immuno-staining. Once stained, the brain sections were examined using light microscopy. A transparent grid of 4 mm

squares was placed over the slide during microscopic examination. The coordinates of the APP positive squares were recorded.

At the time of writing, a simple quantification of the maps of the distribution of axonal injury has been performed. This method has been described previously by Miller et al. (1996). They used a grid to map the lesions on seven representative sections of the brain. They defined the Total Injury Score (TIS) as the number of positive grid squares in the seven representative sections. In the present study, the same score was used, except that in this study the size of each grid square was larger, and more sections (16) were examined. For this reason the TIS in Miller at al.'s study and the TIS in this study are not comparable. By dividing the TIS by the total number of squares which was required to cover all 16 sections of each brain, an estimate was made of the percentage of the brain injured in each animal.

RESULTS

Five experiments were performed as described above. The first two used the initial striker design and were allowed to survive for two hours. It was decided after this that a longer survival time would allow the observable characteristics of the axonal injury to develop more fully. In the final three experiments the survival time was increased to four hours. In the last experiment, a period of hypoxia was imposed on the animal, shortly after the impact. The animal did not survive this period, however.

FORCE AND KINEMATIC RESULTS - Measured kinematic data for each experiment are summarised in Table 2. The impacts were characterised by measured angular accelerations of up to 126 krad/s² and linear accelerations of up to 16 km/s². Impact force measurements were between 6.2 and 8.0 kN, and caused fracture in two cases. In addition to this data, high speed film was taken of all impacts.

Experiment	Striker mass	Charge	F	Head kinematics			Fracture	
				ώ	а	ω	v	
	g		kN	krads-2	kms ⁻²	rads ⁻¹	ms ⁻¹	
Sheep 1	654	'13'	6.2	103	6.7	99	5.4	
Sheep 2	654	'17'	8.5	99	7.0	88	6.4	
Sheep 3	385	'21'	6.6	95	6.6	110	7.5	yes
Sheep 4	385	'21'	5.6	102	9.7	67	6.5	-
Sheep 5	385	'21'	8.0	126	16.0	81	10.7	yes

Table 2 Peak measured kinematic and force values for the five experiments

The force and resultant acceleration traces for each experiment are shown in Figures 5 through 9. Figure 10 shows the linear and angular acceleration components for Sheep 2. The general shape of the acceleration components recorded in this experiment were also characteristic of the other experiments.

In each experiment, a 'bi-phasic' acceleration characteristic was recorded; that is the acceleration phase, which was associated with the duration of the impact force, was followed by a large amplitude deceleration (see Figure 10 for example). These results seemed somewhat anomalous. As the head acceleration is brought about by an impact, it was expected that a large amplitude acceleration phase associated with the impact force would be seen, followed by a much longer, lower amplitude deceleration phase as the head of the animal was brought to rest under the influence of the restraining loads of the neck.

To examine the validity of the measured results, the acceleration data from each experiment was applied to a rigid body numerical (MADYMO) model of the skull-array system. The model consisted of two rigid bodies, representing the head and the array, joined by a bracket joint. The geometry of the model was determined from the stereo-radiographic measurements made for the coordinate transformation process. The displacement predicted by the MADYMO model was compared to the high speed film for each of the five experiments. In every case, the kinematics of the MADYMO model appeared to closely match the kinematics of the array, even up to 30 ms after the impact.



Figure 5 Kinematic data for Sheep 1



Figure 7 Kinematic data for Sheep 3



Figure 9 Kinematic data for Sheep 5



Figure 6 Kinematic data for Sheep 2



Figure 7 Kinematic data for Sheep 4



Figure 10 Orthogonal linear and angular acceleration data for Sheep 2

However, careful examination of the first few milliseconds of motion in the numerical model and in the high speed film revealed that some complex array kinematics occurred during the period of maximum acceleration and deceleration, with the array velocity rapidly changing direction. It appeared that these complex kinematics were due to a combination of relative movement between the array and the skull, and skull bending during the impact. It is likely that the peak recorded acceleration values were associated with these non-rigid-body type motions.

PHYSIOLOGY

Physiological parameters were monitored in each experiment. In every case, the impact was followed by marked physiological changes. The responses were characterised by acute changes and this was sometimes followed by other changes which were delayed and occurred for varying periods throughout the survival period. The short term and long term trends are illustrated on the left and on the right of Figure 11, respectively.

The responses are illustrated for Sheep 1 and Sheep 3, which represent the least and the most severe responses, respectively, observed in the experiments reported on here. Note the large acute change in the intracranial pressure of Sheep 3, which is sustained for the entire survival period, and the decrease in mean arterial pressure. It is also interesting to note that the response of Sheep 1 was an acute decrease in heart rate, whereas the heart rate of Sheep 3 increased acutely.



Figure 11 Physiological response for experiments Sheep 1 and Sheep 3 (a) up until 5 minutes after impact and (b) 2 hours after impact (1 minute averages)

NEUROPATHOLOGY - The principal pathological findings are summarised in Table 3. In Sheep 1 and Sheep 2 there was no skull fracture and on macroscopic examination the brains were essentially normal, apart from focal haemorrhage related to penetration of the subdural intracranial pressure monitor. There was no evidence of subarachnoid haemorrhage or contusion related to the impact. On microscopy, abnormal APP staining was identified in only a few sectors (TIS scores of 9 and 17), and much of this occurred around the track of the pressure monitor. These two animals were impacted using a "13" and "17" charge respectively.

Two animals (Sheep 3 and Sheep 5) sustained fractures. Associated with this was extensive subarachnoid haemorrhage at the left temporal impact site, extending around the brain stem and onto the cerebellum. In both animals fracture contusions occurred in the left temporal and parietal regions (extending through the cortex into digitate white matter) and in Sheep 5 there was a small contre coup contusion in the right parietal region. In both animals microscopy revealed widespread APP abnormalities throughout both cerebral hemispheres (TIS 304 and 135 respectively). These were most prominent around contusions, but also at a distance from vascular injuries, and in Sheep 3 extended into the brain stem. The TIS for Sheep 5 was considerably less than for Sheep 3. However, this animal was subjected to a concomitant hypoxic insult and died only 25 minutes after impact. Given this very short survival time it is

likely that APP abnormalities had not fully developed and had the animal survived for the intended 4 hours, the TIS would have been greater.

Experiment	Survival time	Total Injury Score	Percentage of brain injured
Sheep 1	2 hours	9	1
Sheep 2	2 hours	17	2
Sheep 3	4 hours	304	32
Sheep 4	4 hours	165	19
Sheep 5	25 min	135	16

Table 3 Neuropathological scores

In the remaining case (Sheep 4) extensive subarachnoid haemorrhage, contusion at the impact site and widespread APP abnormalities (TIS 163) similar to those in Sheep 3 and Sheep 5 were seen despite the absence of a fracture. This animal and the preceding two cases were subjected to an impact using a "21" charge.

DISCUSSION

The five experiments presented in this paper represent the development stage of a biomechanical model of axonal injury. As such they do not represent a homogeneous set of experiments from which general conclusions can be made. For instance, fractures occurred in two experiments, which may have altered the energy released into the brain from the impact. In the first two experiments, the survival time was two hours. The duration of this period was increased to four hours in the remaining experiments. In the last experiment, the animal underwent a deliberate period of hypoxia, which may have altered the way in which the cells of the brain were able to deal with the trauma of the impact. All these factors may have had a significant bearing on the observable injury. Nevertheless, some observations have been made regarding these experiments.

In the kinematic simulations, as in the high speed film, it was apparent that non-rigid body motions between the array and the skull were contributing to the measured acceleration. False acceleration measurements associated with skull bending have been encountered by others studying brain injury in an animal model subjected to direct impact (Nusholtz et al., 1984). With the levels of acceleration measured in this study, a fraction of a millimetre of relative movement between the array and the skull would introduce significant errors into the measurement of the acceleration of the head. This effect is unlikely to be unique to this study and care is needed to ensure that the measurements are meaningful in similar types of model. Validation techniques that are being explored for use in this model include the use of additional reference accelerometers rigidly mounted to the skull. By comparing the measured accelerations made using these accelerometers with those predicted from the array accelerations, an indication of the error in the estimation of the skull rigid body acceleration could be made. Additionally, the array itself is being redesigned to make the skull-array attachment stiffer, and to place the sensors closer to the attachment points. This should have the effect of reducing the amplitude of any relative motion measured by the array. Such enhancements should improve confidence in future kinematic results.

Neuropathological examination of these cases revealed a wide spectrum of severity of injury ranging from cases in which the brains were macroscopically normal with little histological evidence of axonal injury, to cases showing extensive subarachnoid haemorrhage, fracture contusions, contre coup contusion and very extensive axonal abnormalities on APP immunostaining. The wide spectrum of injury response is not explained by an inspection of the kinematic data. This may be due, in part, to the potential errors in the kinematic data as discussed above. However it is interesting to note that where there was a focal contusion, due to contact effects from the striker, there was a strong presence of axonal injury and the observable axonal injury was not restricted to the site of impact but was found throughout the brain. Although the number of experiments in this paper is too small to indicate a correlation, the results here imply that some measure of contact phenomena may be important in this model.

SUMMARY AND FUTURE WORK

This paper presents the development of an impact model of axonal injury using sheep which features detailed biomechanical and physiological measurements during the experiment, and a detailed neuropathological description. Five experiments have been reported here, covering a range of conditions and results. Relative motion between the nine accelerometer array and the skull of the sheep was identified as a possibly significant source of error in the measurement the kinematics of the head.

In future experiments, the biomechanical measurements will extend to include the measurement of the dynamic intracranial pressure changes during impact. Additionally, the nine accelerometer array is being redesigned in an attempt to further minimise relative motion between the skull and the array.

This model of axonal injury will be used to study the relationship between impact force, head kinematics and the resulting observed injury. The effect of post-impact physiology will also be examined. A finite element model is also being constructed to examine the relationship between the observed injury and estimated stresses and strains in the brain tissue.

REFERENCES

- Alem, N.H. and Holstein, G.L. 1977, 'Measurement of 3-D motion', *Fifth Annual International Workshop on Human Subjects for Biomechanical Research, Committee Reports and Technical Discussions, New Orleans, Louisiana, USA, October 18, 1977*, Highway Safety Research Institute, Ann Arbor, MI, USA, pp. 242-269.
- Becker, E. 1977, 'Stereoradiographic measurements for anatomically mounted instruments', *Proceedings* of the 21st Stapp Car Crash Conference, Paper 770926, Society of Automotive Engineers, Warrendale, PA, USA, pp. 477-505.
- Blumbergs, P.C., Scott, G., Manavis, J., Wainwright, H., Simpson, D.A. and McLean, A.J. 1994, 'Staining of amyloid precursor protein to study axonal damage in mild head injury', *The Lancet*, vol. 244, no. 8929, pp. 1055-1056.
- Boghani, A.B., Carlson, K.E., Cohen, M.L. and Spencer, R.H. 1989, 'Simulation and calibration of a nineaccelerometer package (NAP) for anthropometric dummies', *Methodology for the Calibration and Data Acquisition with a Six-Degree-of-Freedom Acceleration Measuring Device*, Report no. DOT-HS-807-370, National Highway Traffic Safety Administration, U.S. Department of Transportation, Washington DC, USA, pp. Bi-B1-10.
- DiMasi, F.P. 1995, *Transformation of Nine-Accelerometer-Package (NAP) Data for Replicating Headpart Kinematics and Dynamic Loading*, Report no. DOT-HS-808-282, National Highway Traffic Safety Administration, U.S. Department of Transportation, Washington DC, USA.
- Gennarelli, T.A. 1984, 'Clinical and experimental head injury', *The Biomechanics of Impact Trauma: Proceedings relating to the course given at the International Center for Transportation Studies (ICTS), Amalfi, Italy, May 30 -June 4, 1983*, Eds, Bertil Aldman, André Champon and G. Lanzra, International Centre of Transportation Studies, Amalfi, Italy, pp. 103-115.
- Gennarelli, T.A., L.E., T., Tomei, G., Wiser, R., Graham, D. and Adams, J. 1987, 'Directional dependence of axonal brain injury due to centroidal and non-centroidal acceleration', *Proceedings of the 31st Stapp Car Crash Conference*, Society of Automotive Engineers, Warrendale, PA, USA, pp. 564-574.
- Gennarelli, T.A., Thibault, L.E., Adams, J.H., Graham, D.I., Thompson, C.J. and Marcincin, R.P. 1982, 'Diffuse axonal injury and traumatic coma in the primate', *Annals of Neurology*, vol. 12, no. 6, pp. 564-574.
- Gennarelli, T.A., Thibault, L.E., Tippermann, R., Tomei, G., Sergot, R., Brown, M., Maxwell, W.L., Graham, D.I., Adams, J.H., Irvine, A., Gennarelli, L.M., Duhaime, A.C., Boock, R. and Greenberg, J. 1989, 'Axonal injury in the optic nerve: a model simulating diffuse axonal injury in the brain', *Journal of Neurosurgery*, vol. 71, no. 2, pp. 244-253.
- Hirsch, A.E. and Ommaya, A.K. 1970, 'Protection from brain injury: The relative significance of translational and rotational motions of the head after impact', *Proceedings of the 14th Stapp Car Crash Conference*, Society of Automotive Engineers, New York, NY, pp. 144-151.
- Ljung, C. 1980, 'On scaling in head injury research', *Fifth International IRCOBI Conference on the Biomechanics of Impacts, Birmingham, UK*, Vol. International Research Council on the Biokinetics if Impacts, pp. 209-217.
- Margulies, S.S., Thibault, L.E. and Gennarelli, T.A. 1990, 'Physical model simulations of brain injury in the primate.', *Journal of Biomechanics*, vol. 23, no. 8, pp. 823-836.

IRCOBI Conference - Hannover, September 1997

McLean, A.J. 1996, 'Brain injury without impact?', *Traumatic Brain Injury - Bioscience and Mechanics*, Eds, Faris A. Bandak, Rolf H. Eppinger and Ayub K. Ommaya, Mary Ann Liebert Inc., pp. 45-50.

- Meaney, D.F., Ross, D.T., Winkelstein, B.A., Brasko, J., Goldstein, D., Bilston, L.B., Thibault, L.E. and Gennarelli, T.A. 1994a, 'Modification of the cortical impact model to produce axonal injury in the rat cerebral cortex', *Journal of Neurotrauma*, vol. 11, no. 5, pp. 599-612.
- Meaney, D.F., Smith, D., Ross, D.T. and Gennarelli, T.A. 1993, 'Diffuse axonal injury in the miniature pig: Biomechanical development and injury threshold', *Crashworthiness and Occupant Protection in Transportation Systems presented at the 1993 ASME Winter Annual Meeting, Louisiana, New Orleans, USA, November 28 - December 3 1993,* Eds, J.D. Reid and K.H Yang, American Society of Mechanical Engineers, Applied Mechanics Division, New York, NY, USA., pp. 169-175.
- Meaney, D.F., Smith, D.H., Shreiber, D.I., Bain, A.C., Miller, R.T., Ross, D.T. and Gennarelli, T.A. 1996, 'Biomechanical analysis of experimental diffuse axonal injury', *Traumatic Brain Injury - Bioscience and Mechanics*, Eds, Faris A. Bandak, Rolf H. Eppinger and Ayub K. Ommaya, Mary Ann Liebert Inc., Larchmont, NY, USA, pp. 167-172.
- Meaney, D.F., Smith, D.S., Shreiber, D.I., Rahdert, D.A., Bogen, D.K., Ross, D.T., McIntosh, T.K. and Gennarelli, T.A. 1994b, 'Prediction of experimental diffuse axonal injury severity using macroscopic head motion parameters', 38th Annual Proceedings: American Association for Automotive Medicine, Lyon, France, 21-23 September, 1994, American Association for Automotive Medicine, Des Plaines, III, pp. 345-357.
- Melvin, J.W. and Shee, T.R. 1989, 'Angular acceleration measurement techniques for head impact', *Head Injury Mechanisms: The need for an angular acceleration criterion, Washington DC, October 4, 1989,* American Association for Automotive Medicine, Des Plaines, III, USA, pp. 31-37.
- Mendis, K. 1992, *Finite element modelling of the brain to establish diffuse axonal injury criteria*, PhD Dissertation, Graduate School, Mechanical Engineering, Ohio State University, Columbus, Ohio, USA, 228 pages.
- Miller, R.T., Smith, D.H., Han, X., Xu, B., McIntosh, T.K. and Meaney, D.F. 1996, 'The role of kinetic loading parameters on the severity of diffuse axonal injury in closed head injury', *Impact Head Injury: Responses, Mechanisms, Tolerance, Treatment and Countermeasures, Mescalero, NM, USA, 7 - 9 November, 1996*, Advisory Group for Aerospace Research and Development, in press.
- Mital, N.K. and King, A.I. 1979, 'Computation of rigid-body rotation in three-dimensional space from bodyfixed linear acceleration measurements', *Journal of Applied Mechanics*, vol. 46, no. 4, pp. 925-930.
- Nusholtz, G.S., Lux, P., Kaiker, P. and Janicki, M.A. 1984, 'Head impact response skull deformation and angular accelerations', *Proceedings of the 28th Stapp Car Crash Conference*, Paper 841657, Society of Automotive Engineers, Warrendale, PA, pp. 41-74.
- Nusholtz, G.S., Melvin, J.W. and Alem, N.M. 1979, 'Head impact response of human surrogates', *Proceedings of the 23rd Stapp Car Crash Conference*, Paper 791020, Society of Automotive Engineers, Warrendale, PA, USA, pp. 499-541.
- Ommaya, A.K. and Hirsch, A.E. 1971, 'Tolerances for cerebral concussion from head impacts and whiplash in primates', *Journal of Biomechanics*, vol. 4, , pp. 13-21.
- Ommaya, A.K., Hirsch, A.E., Flamm, E.S. and Mahone, R.H. 1966, 'Cerebral concussion in the monkey: an experimental model', *Science*, vol. 157, , pp. 211-212.
- Padgaonkar, A.J., Krieger, K.W. and King, A.I. 1975, 'Measurement of angular acceleration of a rigid body using linear accelerometers', *Journal of Applied Mechanics*, , pp. 552-556.
- Plank, G., Weinstock, H., Coltman, M. and Lee, H. 1989, Methodology for the Calibration and Data Acquisition with a Six-Degree-of-Freedom Acceleration Measuring Device, Report no. DOT-HS-807-370, National Highway Traffic Safety Administration, U.S. Department of Transportation, Washington DC, USA.
- Saatman, K.E. and Thibault, L.E. 1991, 'Mylenated nerve fiber response to dynamic uniaxial stretch', Proceedings of the 1991 International IRCOBI Conference on the Biomechanics of Impacts, Berlin, Germany, 11-13 September, 1991, International Research Council On Biokinetics of Impacts, Bron, France, pp. 115-126.
- Ueno, K., Melvin, J.W., Li, L. and Lighthall, J.W. 1996, 'Development of tissue level brain injury criteria by finite element analysis', *Traumatic Brain Injury Bioscience and Mechanics*, Eds, Faris A. Bandak, Rolf H. Eppinger and Ayub K. Ommaya, Mary Ann Liebert Inc., Larchmont, NY, USA, pp. 155.
- Ueno, K., W., M.J., Rouhana, M.E. and Lighthall, J.W. 1991, 'Two-dimensional finite element model of the cortical impact method for mechanical brain injury', *Crashworthiness and Ocupant Protection in Transportation Systems - 1991. Presented at the Winter Annual Meeting of the American Society of Mechanical Engineers, Atlanta, Georgia, USA, 1-6 December, 1991*, Eds, T.B. Khalil, C.M. Ni, H.F. Mahmood and A.I. King, American Society of Mechanical Engineers, Applied Mechanics Division, New York, NY, USA., pp. 121-154.