Histopathological variability in 'standardised' spinal cord trauma¹

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SUMMARY Feline spinal cords were traumatised by the weight dropping technique. Five trauma groups were studied (5 g×80 cm, 10 g×40 cm, 20 g×20 cm, 40 g×10 cm, and 80 g×5 cm), each having a 'standardised' injury of '400 g-cm.' The spinal cords were sectioned serially two hours after contusion and examined by light microscopy. Relative to the larger weights falling from lesser heights, the smaller weights falling from greater heights were associated with less haemorrhage, oedema, axonal disruption, and myelin fragmentation as well as a smaller volume of grey matter containing altered anterior horn cells. In all trauma groups the cortical evoked responses disappeared at the time of the injury and did not reappear. Even though each trauma group received a '400 g-cm' contusion, each weight-height combination was associated with differing degrees of histopathological alterations. A plea is made for more accurate quantitation of experimental spinal cord trauma than the 'g-cm' unit.

Since the experiments of Allen in 1911, experimental spinal cord contusion has been quantitated in gram-centimetre (g-cm) units. Trauma is produced by a weight (g) falling a specified distance (cm) through a vertical tube and striking an impounder that is resting on the posterior surface of the spinal cord. The product of the weight and the height from which it falls was expressed as g-cm. The g-cm unit is not precise as various combinations of weights and heights can give the same product; nevertheless, the g-cm unit continues to be used. Certain investigators have used various weight-height combinations interchangeably in their experiments.

The purpose of the present study was to describe the histopathology of the spinal cord and the cortical evoked responses after 400 g-cm 'standardised' trauma, an injury produced by different weights falling from various heights but each giving the same g-cm value.

Materials and methods

Cats were anaesthetised with an intraperitoneal injection of sodium pentobarbitone (35 mg/kg). A tracheotomy was performed and muscular paralysis was obtained with gallium triethiodide. The animals were maintained on a Harvard small animal respirator with the end-tidal pCO₂ maintained between 2% and 4%. Blood pressure was recorded continuously via a catheter in the femoral artery. Temperature was monitored by a rectal probe and kept at approximately 37°C with the help of a heating pad. A screw electrode was placed in the skull over the right somatosensory cortex and the left posterior tibial nerve was stimulated electrically (0.1 ms pulse; 1 V/s). A Biomac 1000 computer was used to analyse the transient cortical potentials. The computer-averaged somatosensory cortical evoked response (CER) was displayed on an oscilloscope and photographed. Cortical evoked responses were obtained before trauma, immediately after trauma, and at one and two hours after the trauma. Laminectomies were performed to expose the T5–6 level of the spinal cord with dura mater intact. Fifteen cats were divided into five trauma groups of three cats each, and the spinal cords were contused with a 400 g-cm injury using the weight dropping

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technique (Albin *et al.*, 1968; Dohrmann *et al.*, 1976): 5 g×80 cm; 10 g×40 cm; 20 g×20 cm; 40 g×10 cm; and 80 g×5 cm. As controls, two cats had laminectomies but were not traumatised. At two hours after the contusion, the cats were perfused with 10% formalin in a retrograde manner via the abdominal aorta, and the exposed portion of the spinal cord was excised. The specimen was embedded in paraffin and the traumatised area was sectioned serially (8 µm) either transversely or longitudinally (two specimens transversely and one longitudinally in each trauma group). Alternate sections were stained with haematoxylin and eosin, Bodian, or Klüver-Barrera stains and examined by light microscopy. All sections were examined and the section showing maximal tissue disruption was chosen for quantitation. Haemorrhage and oedema were graded on a 0 to 4 scale as described in Tables 1 and 2. The cranio-caudal distance over which abnormal anterior horn cells were noted was measured and expressed in millimetres. Quantitation of axonal disruption and myelin fragmentation was carried out by fibre counts or myelin sheath counts at the area of maximal injury. These were expressed as the percentage of abnormal fibres relative to the total number of fibres noted in the section of the posterior columns or of the anterior white matter.

Table 1 Haemorrhage

	Trauma group				
	5 g × 80 cm	10 g × 40 cm	20 g × 20 cm	40 g × 10 cm	80 g × 5 cm
Grey matter	2	3	3	4	3
	2	3	3	4	3
	3	2	2	3	3
White matter	2	2	2	3	2
	0	2	2	3	3
	2	1	2	2	2

0=no haemorrhage; 1=few extravascular erythrocytes; 2=small scattered haemorrhages; 3=large discrete haemorrhages; 4=coalescence of haemorrhages.

Table 2 Oedema

	Trauma group				
	5 g × 80 cm	10 g × 40 cm	20 g × 20 cm	40 g × 10 cm	80 g × 5 cm
	2	3	3	4	3
	1	2	3	4	4
	1	2	3	4	4

0=no oedema; 1=oedema involving entire area of haemorrhage; 2=significant extension of oedema outside of haemorrhagic region; 3=as in 2 and some involvement of white matter; 4=as in 2 and much involvement of white matter.

Results

Although each animal sustained a 400 g-cm contusion of the spinal cord, numerous differences in the histopathology between the various trauma groups were noted.

CONTROL GROUP

No alterations in the histology of the spinal cord were noted in these animals examined two hours after laminectomy.

EXPERIMENTAL GROUP

Haemorrhage

In the 5 g×80 cm trauma group small areas of haemorrhage were noted in the central grey matter and a few scattered areas of haemorrhage were present in the white matter immediately surrounding the grey matter (Fig. 1). Mainly in the grey matter, the haemorrhages increased in number and size in the 10 g×40 cm, 20 g×20 cm and 40 g×10 cm trauma groups respectively (Fig. 2). In the last group coalescence of the haemorrhage was seen centrally. The amount of haemorrhage in the 80 g×5 cm group was less than that at 40 g×10 cm but more than in the other groups (Table 1). Little subarachnoid haemorrhage was present in the 5 g×80 cm and 10 g×40 cm trauma groups while much more subarachnoid haemorrhage was noted in the remaining groups.

Oedema

In the 5 g×80 cm group oedema was present in the region of haemorrhage; however, in the other groups the oedema involved large areas of the

Fig. 1 Photomicrograph illustrating small scattered areas of intramedullary haemorrhage in the 5 g×80 cm trauma group. Bodian ×12.

Fig. 2 Transverse section of spinal cord showing central haemorrhage in the 40 g×10 cm trauma group. Bodian ×18.

spinal cord both proximal and distal to the haemorrhage. The amount of oedematous tissue increased in the 10 g×40 cm, 20 g×20 cm, 80 g×5 cm, and 40 g×10 cm groups respectively. When present, the oedema in the white matter was noted to spread farther than that in the grey matter. (Table 2)

Anterior horn cells

None of the five trauma groups showed a direct relationship between the number of pathologically altered neurones and the amount of injury. However, the distance over which the damaged neurones could be found did correlate somewhat with the degree of trauma (Table 3), but the higher incidence of altered anterior horn cells in the 5 g×80 cm group is not explained.

In the region of the haemorrhage in all groups except 5 g×80 cm and 10 g×40 cm, no recognis-

able tissue remained. Three concentric areas around the haemorrhage were noted in longitudinal section (Fig. 3): (I) Around the haemorrhage in the grey matter only a few neurones were seen. These appeared swollen with very eosinophilic cytoplasm and no nuclei were noted. (II) Around this area was a region of neurones that were shrunken and angular and had deeply basophilic cytoplasm. No nuclei were visible. (III) The outermost region of the grey matter had neurones of normal shape with normal appearing nuclei; however, clumping of the Nissl substance was present. Both proximally and distally beyond region III, the neurones were without pathological change.

Axons

Longitudinal sections of the spinal cords of the 5 g×80 cm and the 10 g×40 cm groups showed fragmentation of the axons of the inner third of

Table 3 Anterior horn cells

Trauma group	Mean length of grey matter containing altered anterior horn cells (mm)	Anterior horn cells (mean)	
		Abnormal	Normal
		(%)	(%)
5 g×80 cm	6.0	90	10
10 g×40 cm	5.4	88	12
20 g×20 cm	6.7	85	15
40 g×10 cm	8.8	75	25
80 g×5 cm	7.0	85	15

Fig. 3 Schematic representation of spinal cord in longitudinal section showing three grey matter regions (I, II, and III) containing altered anterior horn cells both proximally and distal to the central haemorrhage. (The characteristics of cells in each of these regions are detailed in the text.)

the posterior columns. In the 20 g $\times$ 20 cm group a similar number of axons appeared fragmented in the posterior columns (Fig. 4); however, approximately one-third of those of the anterior white matter showed disruption as well. The most severe axonal injury was noted in the 40 g $\times$ 10 cm group where most of the axons of the posterior columns were fragmented and approximately two-thirds of those in the anterior white matter were fragmented (Fig. 5). The number of disrupted axons in the 80 g $\times$ 5 cm group was slightly less

than that noted in the 40 g $\times$ 10 cm group (Table 4).

In transverse section a few swollen axons were noted in the 5 g $\times$ 80 cm group in the lateral white matter immediately adjacent to the grey matter. These increased in number in the 10 g $\times$ 40 cm and 20 g $\times$ 20 cm groups such that in the latter the abnormal axons occupied the entire layer of white matter around the grey matter. This layer of abnormal axons increased in width in the 40 g $\times$ 10 cm and 80 g $\times$ 5 cm groups and was

Fig. 4 Fragmentation of the axons of the medial third of the posterior columns is seen in longitudinal section of spinal cord, 20 g $\times$ 20 cm trauma. Bodian $\times$ 35.

Fig. 5 Approximately 80% of the width of the longitudinally-sectioned posterior columns contains broken axons. 40 g $\times$ 10 cm trauma. Bodian $\times$ 34.

Table 4 Disrupted axons and fragmented myelin

Trauma group	Posterior columns		Anterior white matter	
	Axons	Myelin	Axons	Myelin
5 g×80 cm	35*	65	0	15
10 g×40 cm	25	65	0	0
20 g×20 cm	35	100	30	50
40 g×10 cm	80	100	65	100
80 g×5 cm	75	100	55	100

* Mean percentage of fibres counted in all cats in each group.

noted to involve approximately the medial half of the lateral white matter. Most of the axons in these two trauma groups were shrunken.

Myelin

In both longitudinal and transverse sections, disruption of the myelin was noted to correspond to the areas of axonal damage described above; however, the fragmentation of the myelin extended more anteriorly and posteriorly than the areas of injured axons (Fig. 6, Table 4). In transverse sections of the spinal cord from the 40 g×10 cm and 80 g×5 cm groups, areas of cavitation were seen (Figs. 7 and 8).

Cortical evoked responses

The cortical evoked responses were present in all animals both before and after laminectomy. Immediately after trauma the CERs of all trauma groups disappeared and had not returned two hours after contusion.

Fig. 6 Longitudinal section of contused spinal cord showing disrupted myelin in the medial two-thirds of the posterior columns. 5 g×80 cm trauma. Klüver-Barrera ×16.

Discussion

Schmaus (1890) conducted the first experiments on spinal cord trauma by striking the backs of rabbits and studying the spinal cord. Over the ensuing years various investigators studied experimental spinal cord trauma (Dohrmann, 1972). The first standardisation and quantitation of spinal cord trauma was done in the experiments of Allen

Fig. 7 Myelin sheaths of the lateral white matter are largely intact as seen in this transverse section of spinal cord. 5 g×80 cm trauma. Klüver-Barrera ×130.

Fig. 8 Disruption of myelin of the lateral white matter with early cavitation in transverse section of spinal cord. 80 g×5 cm trauma. Klüver-Barrera ×130.

(1911) where a weight was dropped through a tube to strike the spinal cord. The trauma was described in g-cm units, the product of the weight (g) and the height (cm). Although g-cm is not a true physical unit of measure, it was used to define the degree of trauma delivered to the spinal cord. With the rekindling of interest in experimental spinal cord trauma in the late 1960s the g-cm unit of Allen was again used to describe the injury (Osterholm, 1974). More recently various combinations of weights and heights have been used to produce the same g-cm trauma. Much variability can be seen between the results of certain laboratories. Although some of this may be because different trauma devices and different types of animals are used, some of the variability may well be due to the use of varying combinations of weights and heights which give a superficial appearance of the same amount of trauma.

The light microscopy (Goodkin and Campbell, 1969; Wagner *et al.*, 1969; White *et al.*, 1969; White and Albin, 1970; Ducker *et al.*, 1971; Wagner *et al.*, 1971; Wagner and Dohrmann, 1975; Yeo *et al.*, 1975) and electron microscopy (Dohrmann *et al.*, 1971; Dohrmann *et al.*, 1972) of experimental spinal cord trauma have been described. The central haemorrhage and the alterations in the neurones and myelinated fibres appear to increase with time after trauma. In the present experiment it has been shown that the histopathology of 400 g-cm spinal cord trauma is quite variable. In general the changes are less marked in the trauma groups where a small weight has fallen from a greater height—that is, 5 g×80 cm; 10 g×40 cm—than where a larger weight

has fallen from a lesser height—that is 40 g×10 cm; 80 g×5 cm. After trauma there is tearing of the thin-walled muscular blood vessels within the central grey matter which accounts for the haemorrhagic areas noted therein (Dohrmann *et al.*, 1971; Fairholm and Turnbull, 1971; Dohrmann and Allen, 1975). These blood vessels are prone to disruption by a postero-anterior force, such as used in this experiment, because they are oriented perpendicular to that force (Turnbull, 1972) and because the grey matter offers less support than the white matter. In this experiment the amount of haemorrhage increased from the 5 g×80 cm group to that at 40 g×10 cm. The change in momentum (impulse of the deformation force) in the 40 g×10 cm group would be over 20 times that of the 5 g×80 cm group and a linear relationship exists between impulse and amount of intramedullary haemorrhage (Dohrmann and Panjabi, 1976). This increase in the impulse could account for the variation in the degree of vascular disruption noted in these 400 g-cm trauma groups.

In spinal cord trauma the oedema is mainly vasogenic. Plasma travels from the intravascular space to the extracellular space of the spinal cord via tears in the walls of blood vessels in the grey matter. Green and Wagner (1973) and Griffiths and Miller (1974) noted that the oedema spread centrifugally from the central grey matter to involve the remainder of the grey matter and the surrounding white matter. In this study the amount of oedema appeared to be related to the degree of haemorrhage and, therefore, the amount of vascular disruption.

Neuronal changes were probably due to the

direct effect of the trauma as well as secondary alterations such as ischaemia after the trauma and the ensuing haemorrhage. With increase in the mass of the falling weight, the length of grey matter over which neuronal alterations were noted increased such that it was maximal in the 40 g $\times$ 10 cm group.

Alterations in the axons and myelin were seen first at the junction of the grey matter and the white matter. This can be explained in part by shearing or tensile stresses acting at tissue-tissue interfaces. The axonal and myelin disruption increased in severity from the 5 g $\times$ 80 cm group through those at 40 g $\times$ 10 cm. The energy transferred to the spinal cord in the latter group is approximately 100 times that transferred to the spinal cord in the former group (Dohrmann and Panjabi, 1976). This great differential in the amount of energy absorbed could explain the difference seen in the amount of disruption of axons and myelin. It is interesting that the myelin disruption extends over a larger area than the axonal fragmentation.

The reason that the pathological changes seen in the 80 g $\times$ 5 cm trauma group are usually less than those of the 40 g $\times$ 10 cm group but greater than the other trauma groups is probably related to mechanical factors such as the ratio of the weight to the impounder (Dohrmann and Panjabi, 1976).

Cortical evoked responses have been used to assess the function of the white matter of the spinal cord after trauma (Donaghy and Numoto, 1969; D'Angelo *et al.*, 1973). Absence of the CER correlates with injury to the posterior columns (Allen *et al.*, 1974). Interestingly, in each of the trauma groups in this study, the injury delivered to the spinal cord was sufficient to abolish the CER for at least two hours after contusion.

In general the histopathological variability in 400 g-cm trauma described here indicates that the g-cm unit for quantitating spinal cord trauma is imprecise. Certainly the 5 g $\times$ 80 cm injury is different from that at 10 g $\times$ 40 cm, and so on. The degree of spinal cord trauma should be described in terms of weight and height and not merely in terms of g-cm. Impounder mass should also be noted if an impounder is used. Before comparisons can be made between groups of animals sustaining different amounts of spinal cord trauma, this trauma must be more precisely defined.

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