CONTROLLED CORTICAL IMPACT IN SWINE: PATHOPHYSIOLOGY AND BIOMECHANICS

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ABSTRACT

Investigations of the basic pathological, cellular, and molecular mechanisms of traumatic brain injury (TBI) over the past two decades have been carried out primarily in rodents. Unfortunately, these studies have not translated into improved outcome in patients with TBI. To better model human TBI, a swine model of controlled cortical impact (CCI) was developed. A CCI device was used to generate a focal lesion in 23 anesthetized male Yorkshire swine. In 10 swine CCI parameters of velocity and dwell time were varied to achieve a consistent injury (3.5 m/sec, 400 m/sec, respectively). In 13 swine depth of depression was varied from 9 to 12 mm. Physiological data including heart rate (HR), mean arterial blood pressure (MAP), intracranial pressure (ICP), and cerebral perfusion pressure (CPP) were collected for 10 hours after injury. Following injury, ICP and HR increased above baseline values in all swine with a more pronounced elevation in animals impacted to a depth of depression of 12 mm. An 11 mm depth of depression was found to most closely mimic pathological features of human TBI with edema, infiltration of inflammatory cells, pericapillary hemorrhage, and petechial hemorrhages in the white matter. Injury to a depth of depression of 12 mm resulted in cortical laceration obscuring these features. Immunohistological staining with Neu-N, MAP-2, and Fluoro Jade B revealed evidence of degenerating neurons, axonal disruption, and impending cell death. These results indicate that the swine model of CCI results in a defined and reproducible injury with pathological features similar to human TBI. Physiological parameters after

injury are readily monitored in a setting mimicking conditions of an intensive care unit,

establishing a more clinically relevant experimental model for future investigations.

INTRODUCTION

Traumatic brain injury (TBI) remains a leading cause of death in people younger than 45 years of age in both the United States and Europe (Kraus et al., 1994) and affects up to 2 million victims each year in the United States alone. Patients who survive the initial trauma are frequently left with debilitating neurological deficits. The estimated long-term care costs for TBI exceed 40 billion dollars annually (Fakhry et al., 2004). Investigations of the basic mechanisms and pathology of TBI over the past two decades have been carried out primarily in rodents and have identified many cellular and molecular events associated with traumatic brain injury. Unfortunately, these studies have not improved outcome in patients with TBI. Although there are a number of explanations for the failure in translation from the bench to the bedside in TBI, one possibility is that the current experimental models utilizing rodents may not have sufficient clinical relevance. Several important structural and cellular differences exist between the rodent brain and the human brain. Macroscopically, the rodent brain is lissencephalic whereas the human brain is gyrencephalic. From a biomechanical perspective, the rodent brain is contained in a much smaller cranial vault than the human cranium and has different elastic properties than human brain tissue (Gefen et al., 2003; Thibault and Margulies, 1998). Histologically, rodents have one glial cell for every neuron, whereas in humans the ratio is 10 glial cells for every neuron (Finnie and Blumbergs, 2002). As a result, there are sparse white matter tracts within the rodent brain, while the human brain has large white matter tracts, which are often a site of significant brain injury after trauma (Finnie and Blumbergs, 2002). Rodent experimental TBI protocols also have little similarity to protocols used in the care of patients with TBI.

Following the initial insult, rodents recuperate from experimental injury in a cage without support while most patients with severe TBI are treated in an intensive care environment with intubation, sedation, controlled ventilation, multimodal physiological monitoring, and rigorous control of intracranial pressure and cerebral perfusion. Collectively, these issues limit the translational potential of a rodent experimental model of TBI.

Conversely, the swine brain has many similarities to that of humans. Its gyrencephalic cortical structure, its growth and development, and its glial cell to neuron ratio are similar to that of the human brain. Its constituents are also very similar, with identical myelin levels and water content. Biomechanically, the elastic and viscous behavior of the swine brain as well as its frequency-dependent complex sheer modulus is similar to that of the human (Thibault and Margulies, 1998). The relatively large size of the swine allows physiological monitoring in an environment similar to the intensive care unit. All these factors suggest that the swine may provide a promising experimental model to replicate human traumatic brain injury. The purpose of this study was to develop a clinically-relevant large animal model of TBI using controlled cortical impact (CCI) with monitoring in an intensive care unit environment.

METHODS

Preparation of the Swine

Male Yorkshire swine weighing from 38 kg to 42 kg were studied in the large animal surgery/intensive care lab (Figure 1A). The swine were housed in the UCSF animal care facility for at least 4 days before any experimental procedure and allowed

free access to food and water until 12 h before anesthesia was induced. After intubation,

anesthesia was maintained with a fentanyl infusion (1 mcg/kg/h) and inhaled isoflurane

(MAC, 1.2-2%). The swine were paralyzed initially with 0.05 mg/kg of pancuronium and

received supplemental bolus injections as needed to maintain 100% peripheral-nerve-

twitch suppression. A femoral venous catheter was placed for delivery of maintenance

fluids. Central venous access was achieved via the internal jugular vein and a Swan-Ganz

catheter placed to measure cardiac indices. Femoral artery catheterization was performed

for continuous blood pressure measurement and access for arterial blood gas analysis.

The bladder was catheterized and urine output recorded. A ventilator (Narkovet 2,

Drager, Telford, PA, USA) was used to deliver inhalation anesthetics and controlled

ventilation. Inspired oxygen concentration and end-tidal CO_2 were monitored

continuously. Additional monitoring included EKG and pulse oximetry. All incisions for

monitoring access were closed and appropriately dressed. Body temperature of 38.5°C+/-

1.0°C was maintained by using a forced air warmer (BAIR Hugger, Augustine Medical,

Eden Prairie, MN, USA). Physiological data from the swine was collected continuously

on a computerized data acquisition system designed and built for the lab by San

Francisco Industrial Software (San Francisco, CA, USA).

The head of the swine was placed in a modified large animal stereotactic frame

(David Kopf Instruments, Tujunga, CA, USA) (Figure 1B) with custom made skull pegs

affixed to the zygoma to prevent movement at the time of impact. A 15-mm burr hole

was made on the right side of the skull, 7 mm anterior to the coronal suture and 3 mm

lateral to midline over the frontal lobe to expose the dura for impact (Figure 2). The bone

was carefully removed so as not to disturb the dura and underlying brain tissue. A

fiberoptic intracranial pressure monitor (Integra Lifesciences, Plainsboro, NJ, USA) was

placed in a 2-mm burr hole on the left 5 mm caudally to the coronal suture for continuous

monitoring of intracranial pressure (ICP) (Figure 2).

Controlled Cortical Impact

The controlled cortical injury device (Figure 3A) was built by the Bioengineering

Department at the Medical College of Virginia. The impactor consists of a small-bore,

double-acting, pneumatic cylinder with a piston to which is attached an impactor tip. The

cylinder is mounted on a rigid crossbar and is adjusted to an angle of 6 degrees in the

vertical axis over the head. A 15-mm impactor tip with a beveled edge is attached to the

lower end of the threaded rod. The impactor tip was modified to achieve a completely

beveled edge to limit tearing of the dura at impact (Figure3B). The upper end of the rod

is attached to the transducer core of a linear voltage differential transducer (LVDT). The

velocity of the impactor is controlled by fine tuning the flow of high-pressure gas to the

pneumatic piston and can be adjusted between 1.0 and 6.0 m/s. The velocity is calculated

from the time/displacement curve measured by the LVDT and recorded by a

computerized data acquisition system. The dwell time may also be controlled.

Following preparation of the swine the head with the stereotactic frame was

placed in the CCI device. For the CCI model determination of the reference point from

which the depth of depression is set is crucial for creating a reproducible injury. This is

particularly difficult in a swine brain with its thick calvarium, gyrencephalic structure,

extensive CSF space, and a curved dural surface (Figure 4A). Because of these variables

the reference point cannot be set visually. Therefore, we developed a physiologic method

to determine the reference point. The output from a Camino ICP (Integra Lifesciences, Plainsboro, NJ, USA) was displayed at high-resolution on a monitor to allow the measurement of small changes in pressure as the impactor tip touches the dura (Figure 4B). The impactor tip was adjusted to the level at which repeated manual strikes resulted in a rise of ICP of 1 mmHg above peak ICP values during inspiration (Figure 4B).

Experimental Protocol

A total of 23 pigs were studied in these experiments. In 10 animals velocity, depth of depression, and dwell time were varied from 2-4 m/sec, 2 to 10mm, and 50 to 400 msec, respectively, in order to achieve a grossly visible injury. Velocity and dwell time were set at the level at which a grossly evident injury resulted consistently (3.5 m/sec and 400 msec). In 13 swine, depth of depression was varied, to a depth of 9mm (n=3), 10mm (n=3), 11mm (n=4) and 12mm (n=3). Histological studies were performed in these 13 animals. Physiological data including heart rate (HR), mean arterial blood pressure (MAP), intracranial pressure (ICP), and cerebral perfusion pressure (CPP)) were collected for 10 hours after injury in 10 of these13 animals.

Histological Studies

After completion of monitoring, the swine received an overdose of pentobarbital. The brain was carefully removed, photographed, and then post-fixed in 10% formalin for 10 days followed by cryoprotection in 20% sucrose. The formalin-fixed tissue was then sectioned into 50 micron thick coronal sections for Nissl staining using a freezing microtome (Leica, Wetzlar, Germany). Coronal sections of the frozen tissue were cut at

14 micron thickness for immunohistochemistry using a cryostat (Leica , Wetzlar,

Germany). Commercially available antibodies to NeuN and MAP-2 were used to identify

nuclear-specific protein and microtubule associated protein-2, respectively, in neurons.

For NeuN and MAP-2 staining, the sections were first fixed in 4%

paraformaldehyde/0.1M phosphate buffer (PB). Sections were then washed in 0.1M PB

(pH = 7.4), followed by 2 hours incubation in blocking solution (100 mM PB, 0.1%

Triton-X 100, 3% bovine serum albumin and 2% sheep serum). The sections were then

exposed to primary mouse monoclonal antibody raised against NeuN (Chemicon

International, Temecula, CA, USA; 1:500) or MAP2 (Chemicon International, Temecula,

CA, USA; 1:200) for 24 hours at room temperature, diluted in blocking solution.

Following a wash in 0.1M PB, the sections were incubated for 2 hours in biotinylated

sheep anti-mouse secondary antibody (1:200, Amersham Biosciences, Buckinghamshire,

UK). After washing in 0.1M PB, both NeuN and MAP2 immunoreactivities were

detected using the avidin-biotin system (Elite Vectastain ABC kit; Vector Laboratories,

Burlingame, CA, USA) with 0.05% diaminobenzidine-tetrachoride (DAB Fast; Sigma,

St. Louis, MO, USA) as the chromogen. Controls for immunohistochemistry consisted of

omission of the primary antibody.

To localize neuronal degeneration, coronal sections adjacent to the injury were

stained using the fluorochrome, Fluoro-Jade B. Sections were then washed in 0.1M PB

(pH=7.4) and stained with Fluoro-Jade B (Histo-chem Inc., Jefferson, AR, USA) as

described by Schmued et al (Schmued and Hopkins, 2000).

RESULTS

Depth of depression and Physiological Parameters

Intracranial pressure immediately following impact is depicted in Figure 5. The rise in normalized ICP immediately after impact was higher at a 12 mm depth of depression than in the other groups, suggesting a qualitatively different initial ICP response at this depth of depression. This trend towards a higher elevation of ICP in pigs impacted to 12 mm was maintained throughout the duration of the experiment as seen in Figure 6. In pigs impacted from 9 mm to 11 mm, ICP increased 70% above baseline values at 10 hours post-injury, while pigs impacted to 12 mm had ICP values 117% above baseline values. Data depicting mean HR, MAP, and CPP for10 hours following cortical impact to various depths of depression are shown in Figure 7. A trend towards a more rapid heart rate is seen in animals impacted from 9 mm to 11mm (27% above baseline), but is more pronounced in the swine impacted to a depth of 12mm (62% increased above baseline), further indicating a qualitatively different response at this level of injury. MAP and CPP trended downwards in both groups.

Gross pathological features

All animals had surface contusions and subarachnoid hemorrhage at the site of impact. Two of the three animals that were impacted to a 9 mm depth of depression had petechial hemorrhages confined to the impacted hemisphere. Two out of the three animals impacted to a 10 mm depth of depression had midline shift (an indicator of significant cerebral edema), as well as petechial hemorrhages. Animals impacted to an 11 mm depth of depression had petechial hemorrhages and hemorrhage in the white

matter and grey-white junction, consistent with axonal injury. All swine undergoing impact to a 12 mm depth of depression had a cortical laceration at the impact site and underlying intraparenchymal hemorrhage (Figure 8).

Histological features

The morphologic features seen on Nissl staining following CCI, displayed the typical features seen in contusions regardless of the depth of depression. These included edema, infiltration of inflammatory cells, and pericapillary hemorrhage. However, as the depth of depression of the impact increased, these pathologic features tended to increase in severity. At a 9 mm depth of depression, perivascular hemorrhage, edema, increased cellular infiltrates, and a poor neuronal organization were noted distal to the site of impact (Figure 9C and 9D). Decreased Nissl staining in the grey-white junction secondary to a decrease in neuronal density in the area of the contusion was also evident. At a 10 mm depth of depression, these findings were present along with vascular congestion and a more pronounced decrease in neuronal density (Figure 9E-9F). At 11 mm depth of depression the direction of the force of impact was evident by the decreased Nissl staining in the sulci distal to the impact site (Figure 9G-H). In addition, petechial hemorrhages in the white matter at the site of impact were seen. At the 12 mm depth of depression, the typical features seen in contusions were obscured by lacerations of the brain surface (data not shown).

Immunohistochemsitry

Subsequent immunohistochemical studies were performed on the brains of swine that sustained CCI to an 11 mm depth of depression. This depth of depression was chosen for analysis because the histological injury patterns on Nissl staining were felt to optimally replicate patterns of injury found in human TBI without producing frank laceration of the cerebral tissue. MAP-2 immunostaining in the brain of swine that did not undergo CCI demonstrated the fine slender dendrites of neurons with homogeneous staining of MAP-2 radiating throughout the entire cerebral cortex. In animals that underwent CCI, MAP-2 immunostaining in the non-traumatized hemisphere was similar to that of control animals (Figure 10A, C). However in the impacted hemisphere, the area surrounding the site of CCI was either devoid of MAP-2 immunostaining (Figure 10B) or else displayed MAP-2-positive fragments of damaged neuronal structures (Figure 10D). NeuN immunostaining in the brain of swine that did not undergo CCI demonstrated homogeneous staining of a dense layer of neuronal nuclei with lighter staining cytoplasm throughout the cortex. In animals that underwent CCI, NeuN immunostaining in the non-traumatized hemisphere was similar to that of control animals (Figure 10E). However, in the hemisphere ipsilateral to impact, the area surrounding the site of CCI displayed a significant decrease in NeuN immunostaining (Figure 10F), suggestive of neuronal loss. Fluro-Jade B has been shown to have great affinity for degenerating neurons and stains the cell body, dendrites, axons, and axon terminals (Schmued and Hopkins, 2000). Fluro-Jade B staining in the brain of swine that did not undergo CCI had minimal background staining and no neuronal staining. In animals that underwent CCI, Fluoro-Jade B staining in the non-traumatized hemisphere was similar to

that of control animals (Figure 10G). However in the hemisphere ipsilateral to CCI, the area surrounding the site of impact showed numerous Fluoro-Jade B positive neurons with prominent staining of cell bodies, dendrites, and axons (Figure 10H).

DISCUSSION

Model development

This report describes the development of a swine model of CCI. The physiological, gross pathological, histological, and immunohistochemical changes observed following injury at varying depths of depression are detailed. The swine may be a particularly well-suited species for a cortical impact model of TBI because of the many similarities between its brain and the human's. Moreover, many of the physiological systems of the pig are well characterized including its renal, respiratory, and cardiac function (Hannon et al., 1990). As a larger animal, it can be fully instrumented with the same devices used to monitor humans after traumatic brain injury in an environment similar to a modern intensive care unit, placing experimental studies in a relevant clinical context. .

In developing the current model technical issues emerged. The first, and most difficult to overcome, was setting the appropriate reference point from which depth of depression is determined. Unlike the rodent brain which is lissencephalic and uniform in configuration, the swines' brain represents a more complex structure with variability in the curvature of the dura, the gyral and sulcal pattern, and the size of the subarachnoid space. Failure to account for these factors introduces significant variability to the CCI model. To surmount these issues we developed a physiologic method of setting the reference point by measuring changes in ICP resulting from displacement of the dura by

the impactor. Although our use of a 1 mm Hg rise in ICP above peak inspiratory values is arbitrary, its employment resulted in a reproducible injury at each particular depth of depression. Another technical issue that needed to be addressed was rigid fixation of the swine's head in a stereotactic frame. We were concerned that standard ear bars and snout support would fail to prevent movement of the head during impact. To solve this problem, custom made pegs were designed with two points of fixation on either side of the skull (Figure 1B) providing rigid fixation of the cranium during impact.

Defining the appropriate depth of depression was another important variable. Several factors were taken into account in determining the depth that best produced features of human brain injury. Impact to a depth of depression of less than 9 mm did not produce significant cortical injury, whereas a depth of depression to 12 mm produced an excessive injury with laceration of the cortical surface. At depths of depression from 9 mm to 11 mm, the physiological parameters of HR, MAP, ICP, and CPP after impact were similar between groups. Importantly, the gross pathological features in this range of injury were similar to those described in human brain contusions. For example, the surface contusions, subarachnoid hemorrhage, petechial hemorrhages, and hemorrhage in the white matter of our injured swine brains resemble contusions in the human brain which are found on the crests of gyri and in the deeper layers of the cortex, and may involve the adjacent white matter (Graham, 2002). The histological changes following CCI appeared to increase in severity with increasing depth of depression, and included perivascular hemorrhage, petechial hemorrhages in the white mater, edema, vascular congestion, increased cellular infiltrates, and a poor neuronal organization with a pronounced decrease in neuronal density. These findings correspond to the pathological

characteristics of contusions in humans which also include perivascular hemorrhage,

vascular margination, ischemic changes in neurons, and non-hemorrhagic necrosis

(Graham, 2002).   Importantly, pathological features such as white mater petechial

hemorrhages and extensive subarachnoid hemorrhage seen in our swine model are not

found in rodent models of CCI due to the limited white mater and smaller subarachnoid

space in the rodent brain (Finnie and Blumbergs, 2002).

Immunohistochemistry

In swine undergoing CCI to a depth of 11mm, immunohistological staining with the

markers MAP-2, Neu-N, and Fluro-Jade B was performed. MAP-2, a cytoskeletal

protein, is a well-established sensitive marker for neuronal impairment after various types

of brain injury, including ischemia (Dawson and Hallenbeck, 1996; Kitagawa et al.,

1989) and neurotrauma (Brodhun et al., 2001). In contusions, MAP-2 immunostaining

has been shown to be decreased or lost in and near the contusion site (Saatman et al.,

1998), and varies in intensity in relation to the severity and time course of injury (Folkerts

et al., 1998). Decreased MAP-2 staining is correlated with neuronal death(Hicks et al.,

1995) and can be used to assess neuronal loss before morphological changes become

evident (Taft et al., 1992). In our current swine model, the absence of MAP-2 staining in

regions surrounding the site of impact suggests neuronal damage and impending neuronal

death near the site of focal injury.

NeuN is a well-studied DNA-binding, neuron-specific protein, expressed in post-

mitotic neurons (Mullen et al., 1992). The loss of NeuN staining in the region

surrounding impact site of the injured swine provides additional evidence of impending cell death.

Fluoro-Jade B is a novel fluorescent marker that has been shown to have great affinity for degenerating neurons and stains the cell body, dendrites, axons, and axon terminals (Schmued and Hopkins, 2000). Studies have shown that Fluoro-Jade B stains both apoptotic and necrotic neurons undergoing a variety of neurotoxic insults, including injury by kainic acid and oxygen-glucose deprivation (Bonde et al., 2002). Although its exact chemical identity is unknown, it has been postulated that like its sister compound, Fluoro-Jade, Fluoro-Jade B is a specific marker of general neuronal death (Sato et al., 2001; Schmued and Hopkins, 2000). In our swine model of CCI, the prominent staining with Fluro-Jade B of the cell bodies, dendrites, and axons of neurons in the area surrounding impact suggests that these neurons undergo degeneration soon after injury. Together these results demonstrate the feasibility of using these well-established markers in the swine.

Why another model?

Traumatic brain injury has been modeled in animals for over 125 years raising the important question of why another model of TBI is needed. All the experimental models of TBI fall into two broad categories: (1) those in which the calvarium is closed when the injury is delivered, and (2) those in which the calvarium is open so that the brain is injured by direct deformation. In the closed calvarium models injury patterns tend to be more diffuse than focal. Impact in these models is accomplished by a variety of means, including firing a captive bolt pistol (Finnie and Blumbergs, 2002), impacting with a

weighted pendulum, or dropping a weight directly onto the surface of the skull. While these models provide good experimental paradigms of diffuse injury they do not provide a consistently reproducible focal trauma to the brain.

The open calvarium models more closely model focal brain injury. In these experimental models the brain is directly deformed by a force that is rapidly deployed against the exposed dura, producing an injury that is more focal and localized in nature. One frequently used model of this type is the fluid percussion model, in which the energy of a falling pendulum is transferred to a fluid-filled cylinder in direct continuity with the brain (Finnie and Blumbergs, 2002). This model has been used in swine (King et al., 2004), but the neuropathology following injury in this species has not been thoroughly characterized. One criticism of the fluid percussion model is that an indeterminate amount of energy is transferred to the space between the dura and the skull (Lighthall et al., 1989). This may be particularly problematic in swine, where the large subarachnoid space may absorb some of the impact energy, increasing experimental variability. In addition, this technique produces more diffuse pathological changes (Dixon et al., 1988; Dixon et al., 1987), suggesting that this model is more appropriately a model of brainstem injury.

Controlled cortical impact is likely the technique which reproduces human focal TBI most accurately. This model of traumatic brain injury uses a known impact interface, and a measurable, controllable impact velocity that creates a defined cortical deformation. A quantitative relationship has been established between important biomechanical variables, such as force, velocity and deformation, and the magnitude of tissue damage and functional neurological impairment. Two other groups have recently published

reports of impact injury in the swine. Alessandri and colleagues (Alessandri et al., 2003) used a custom-built CCI device to generate a focal brain injury and demonstrated local changes in regional cerebral metabolic parameters including brain tissue oxygen, glucose, lactate, and glutamate. Duhaime and colleagues have studied piglets of different age in which, unlike the CCI model, the impact piston is rested directly on the surface of the dura and then driven downward into the brain, causing a focal injury from displacement rather than impact (Duhaime et al., 2000). The resulting MRI features of this injury are similar to those found in human TBI (Grate et al., 2003). Taken together with the current study, these results demonstrate the potential of focal injury models in swine to provide data that may translate to the clinical realm. The CCI model described here provides an ideal experimental platform to study important issues in human TBI, such as the optimal placement and use of advanced neuromonitors, the efficacy of new pharmacologic agents, and the evaluation of new treatment strategies.

Limitations of the model

While our CCI model may be clinically relevant, it has certain limitations. First, the clinical equipment necessary to replicate an ICU is expensive. Second, because the physiologic response of the injured swine is similar to injured humans, they require intensive management including ventilatory and physiologic support. Therefore, clinically trained personnel are required to continuously monitor and treat the swine after injury. A further issue is the lack of functional neurological outcome measures in the swine. Lastly, many of the molecular and immunohistochemical markers and reagents

established in rodents have not been used in the swine and will require further evaluation

as we have done for MAP-2, NeuN, and Fluoro Jade.

CONCLUSIONS

We present a new model of CCI in the swine that produces a defined and

reproducible brain injury with pathological features similar to those of human focal TBI.

This large animal model offers the opportunity to collect physiological data following

brain injury in an environment similar to the ICU. It is hoped that this may provide data

translatable to the clinical realm and mark the beginning of a new direction in

neurotrauma research.

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FIGURE LEGENDS

1. (A) The large animal surgery/intensive care laboratory at UCSF with extensive physiological monitoring capabilities similar to a standard intensive care unit. **(B)**

The large animal stereotactic frame modified with 2 skull pegs attached to each arm allowing for rigid fixation of the head to prevent movement during impact.

2. Schematic of the swine skull showing the location of the burr hole for the injury impact site in the right frontal lobe. Location of the intracranial pressure monitor posterior to the coronal suture on the left side is also shown.

3. (A) Pneumatic CCI device is shown with the stereotactic frame (**Fig 2B**) in position for impact. (**B**) Detail of the 15 mm impactor tip and modified beveled edge used to help prevent tearing of the dura at impact.

4. (A) Schematic representation of a coronal view of the swine brain with its gyrencephalic structure (grey) and large subarachnoid space presenting a complex structure for impact. (**B**) The reference point from which depth of depression is calculated is set at the level at which repeated manual dural strikes with the impactor tip result in a 1 mm Hg rise in intracranial pressure above peak inspiratory values (arrow). For further details see Methods.

5. Normalized intracranial pressure immediately following controlled cortical impact increased slightly with increasing depth of depression from 9 mm to 11 mm. Much higher relative values are noted immediately after impact to 12 mm depth of depression.

6. Intracranial pressure for 10 hours after controlled cortical impact showing a trend of increased intracranial pressure in all groups with higher values in swine impacted to a 12 mm depth of depression.

7. Mean heart rate (HR), mean arterial pressure (MAP), and cerebral perfusion pressure (CPP) for 10 hours following controlled cortical impact. A trend

towards a more elevated heart rate is seen in swine undergoing impact to a 12 mm depth of depression than to other depths.

8. **Gross Pathology for 9 mm to12 mm depth of depression.** Coronal sections reveal increased subarachnoid hemorrhage and focal contusion in the injured hemisphere as the depth of depression was increased from 9 mm to 11 mm. Cortical laceration and intraparenchymal hematoma (arrow) occurred when the depth of depression was further increased to 12 mm.

9. Increasing cytoarchitectural disruption is observed with increasing depth of depression. Photomicrographs of Nissl staining on the cortex and sulcus of control brain showing normal cytoarchitecture (**A, B**), Brain impacted with depth of depression of 9mm (**C, D**), 10mm (**E, F**) and 11mm (**G, H**) showing increased loss of the normal cellular architecture.

10. **Histochemical analysis of the uninjured contralateral side and the injured (CCI impacted) ipsilateral side.** Animals were impacted with 11 mm depth of depression. Representative coronal sections with MAP-2 immunostaining after impact on the uninjured side (A) and injured side (B) demonstrating loss of MAP-2 reactivity on the injured side (Scale bar 1000μm). A higher magnification of MAP-2 staining is shown in panels **C** and **D** axonal disruption in the injured cortex (Scale bar 100μm). NeuN immunohisochemistry of uninjured (**E**) and injured (**F**) side reveals loss of normal neuronal architecture (Scale bar 500μm). Fluoro-Jade B positive cells in the injured hemisphere indicate degenerating neurons (**H**). No degenerating neurons are observed on the uninjured side (**G**) (Scale bar 100μm).

Normalized ICP by depth of depressia

Lapsed time (hrs)

9mm
10mm
11mm
12mm

HR (bpm)   MAP (mm Hg)   CPP (mm Hg)

9 mm depth

10 mm depth

11 mm depth

12 mm depth

time (hours)

impact

CORTEX

SULCUS
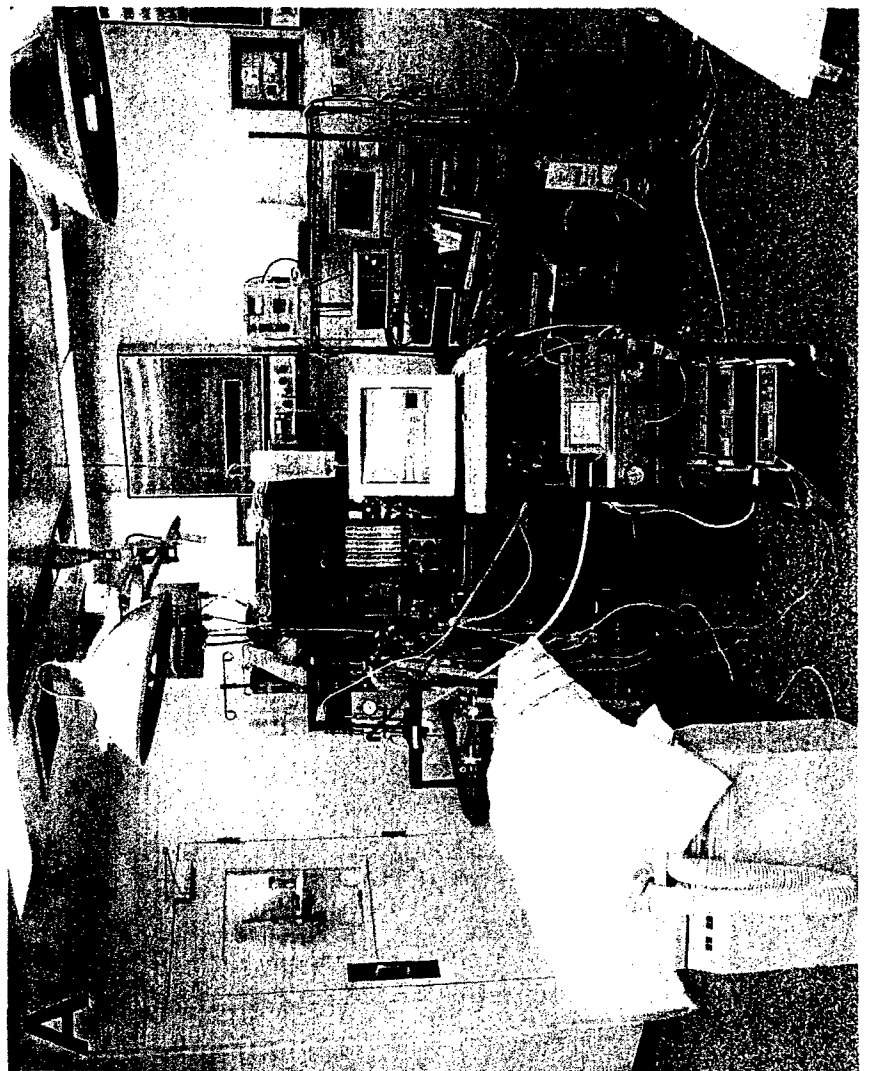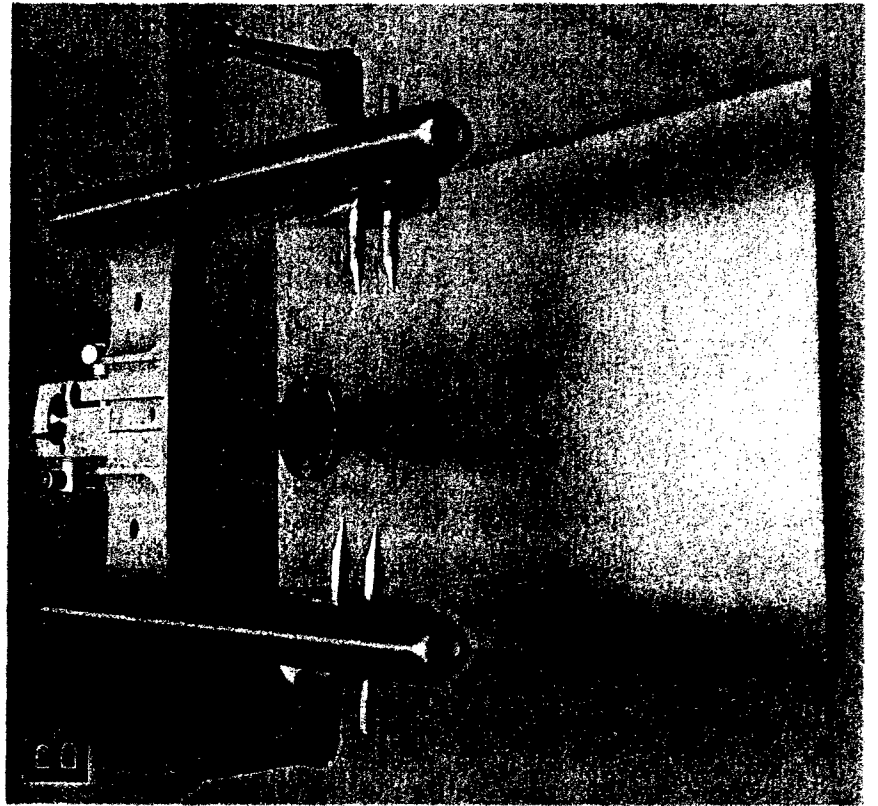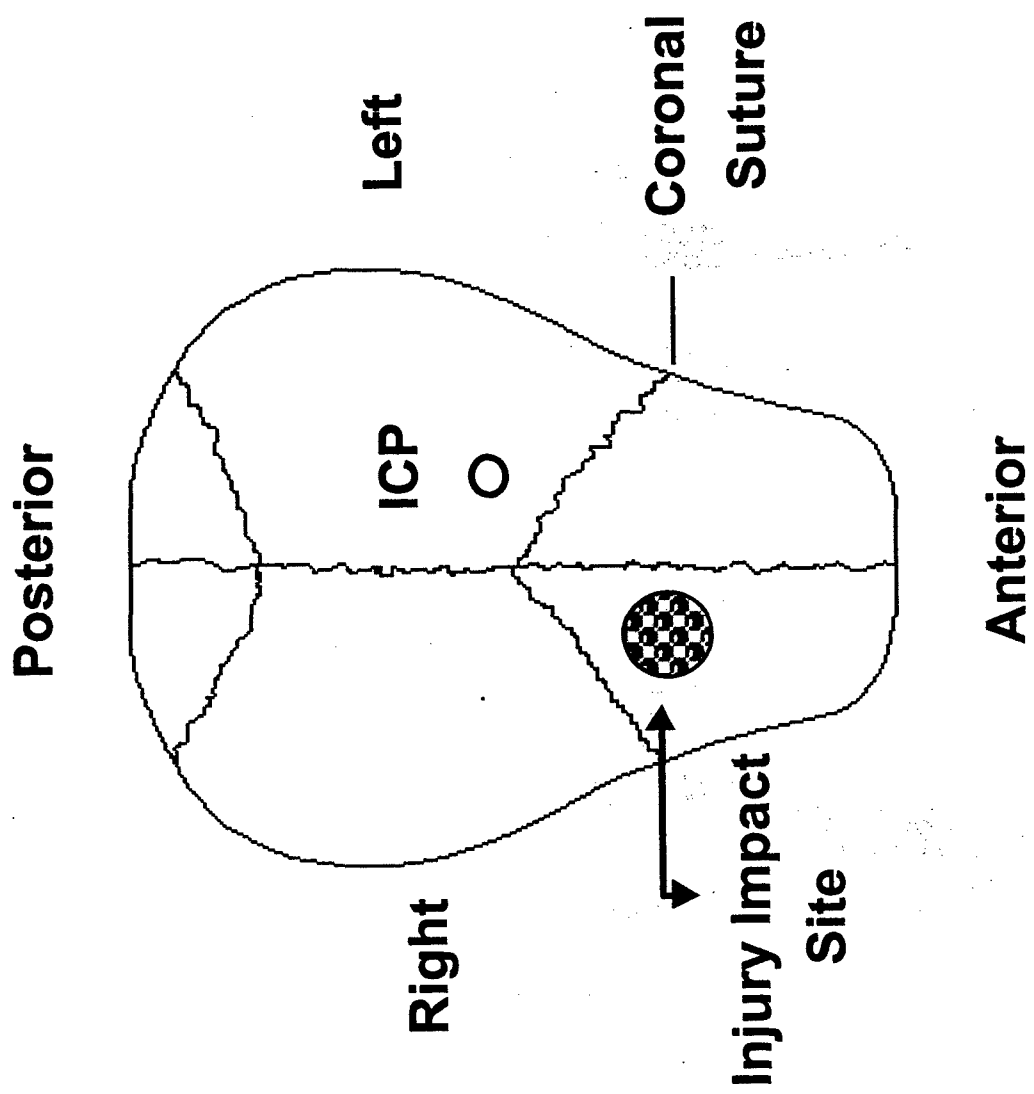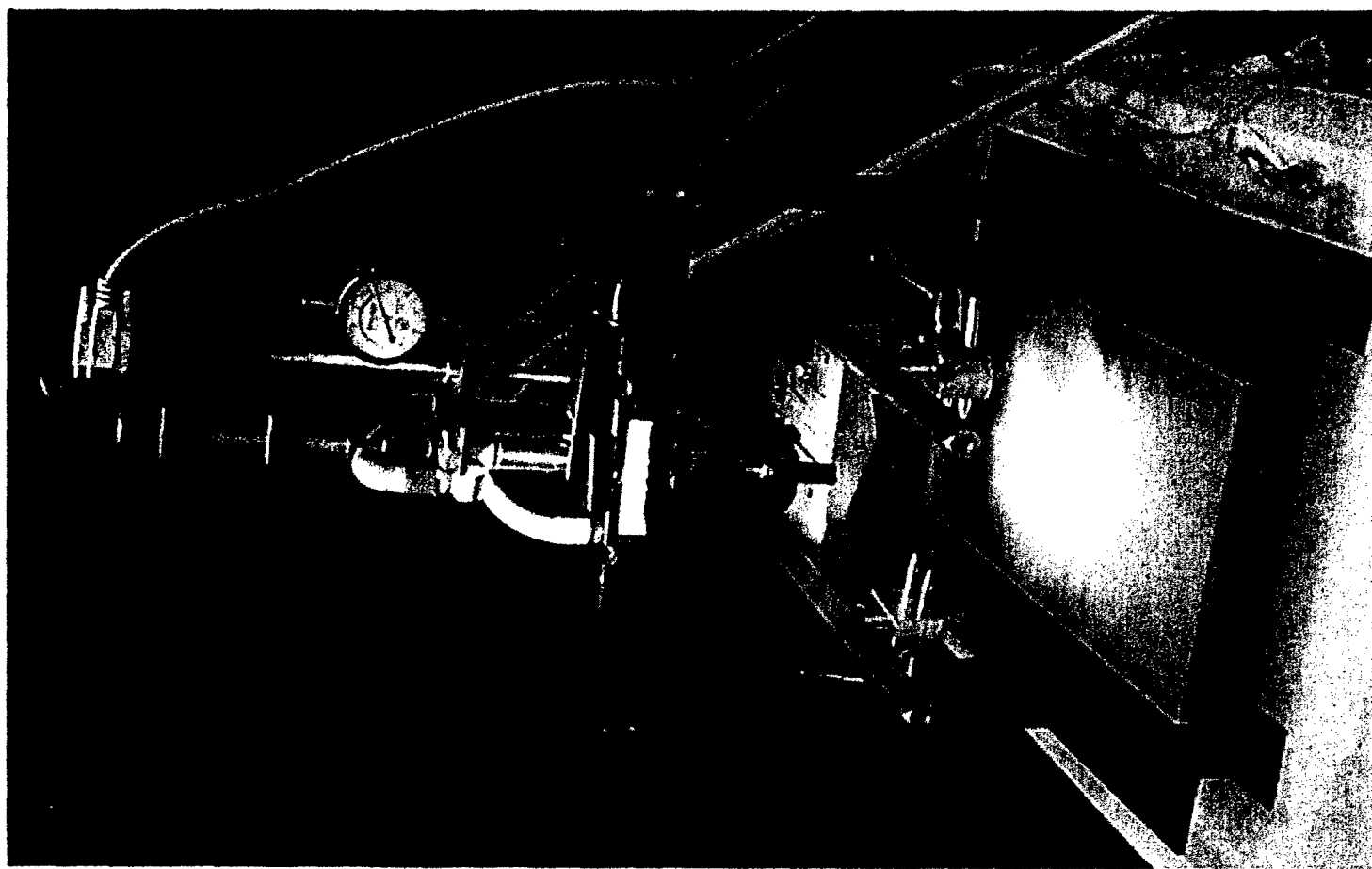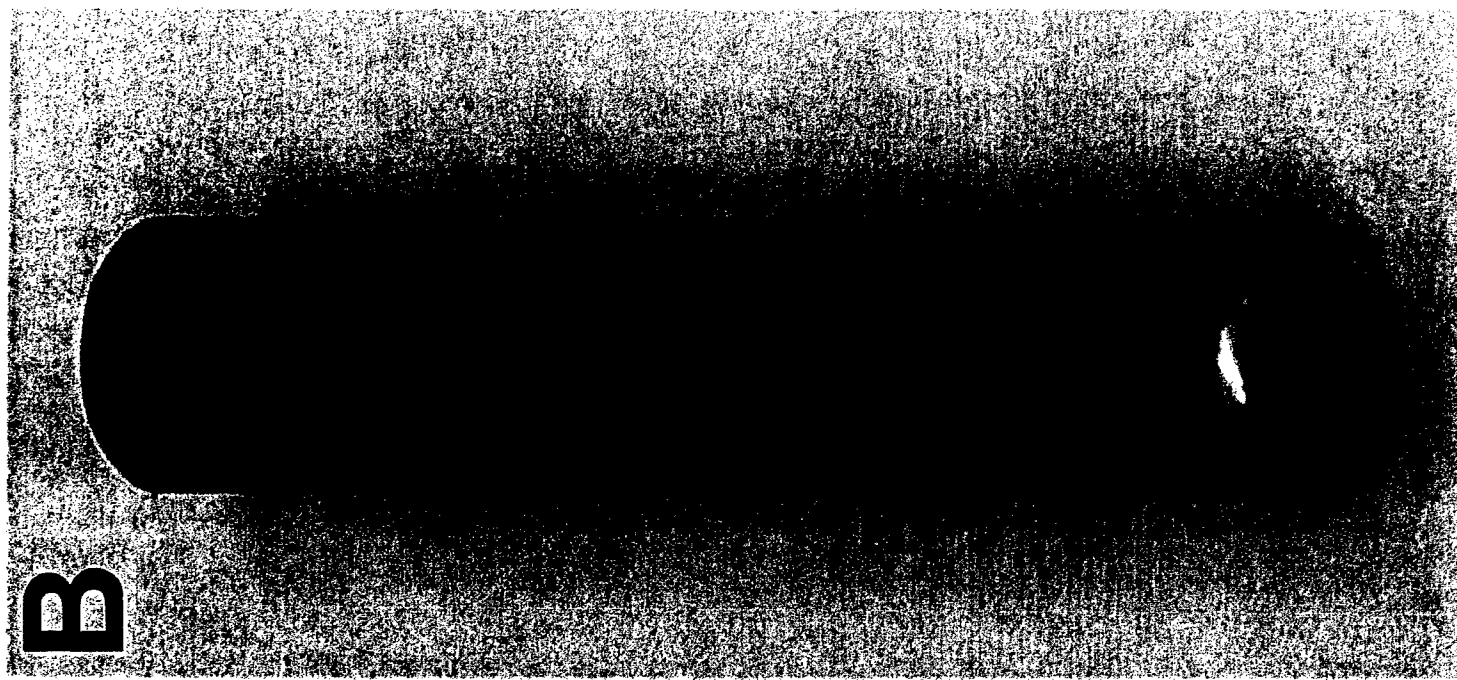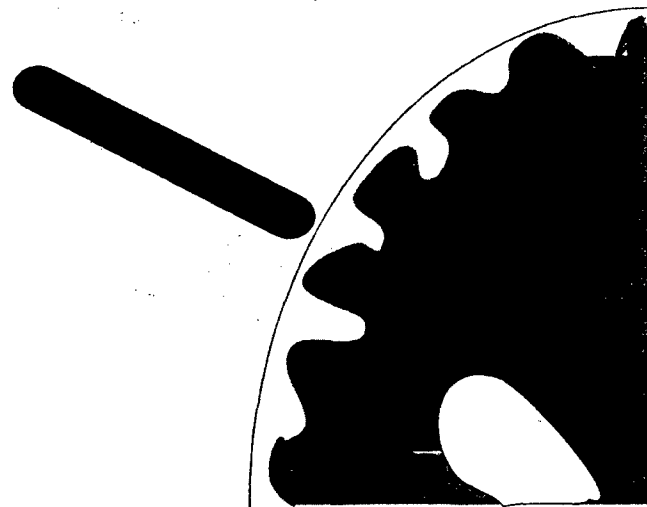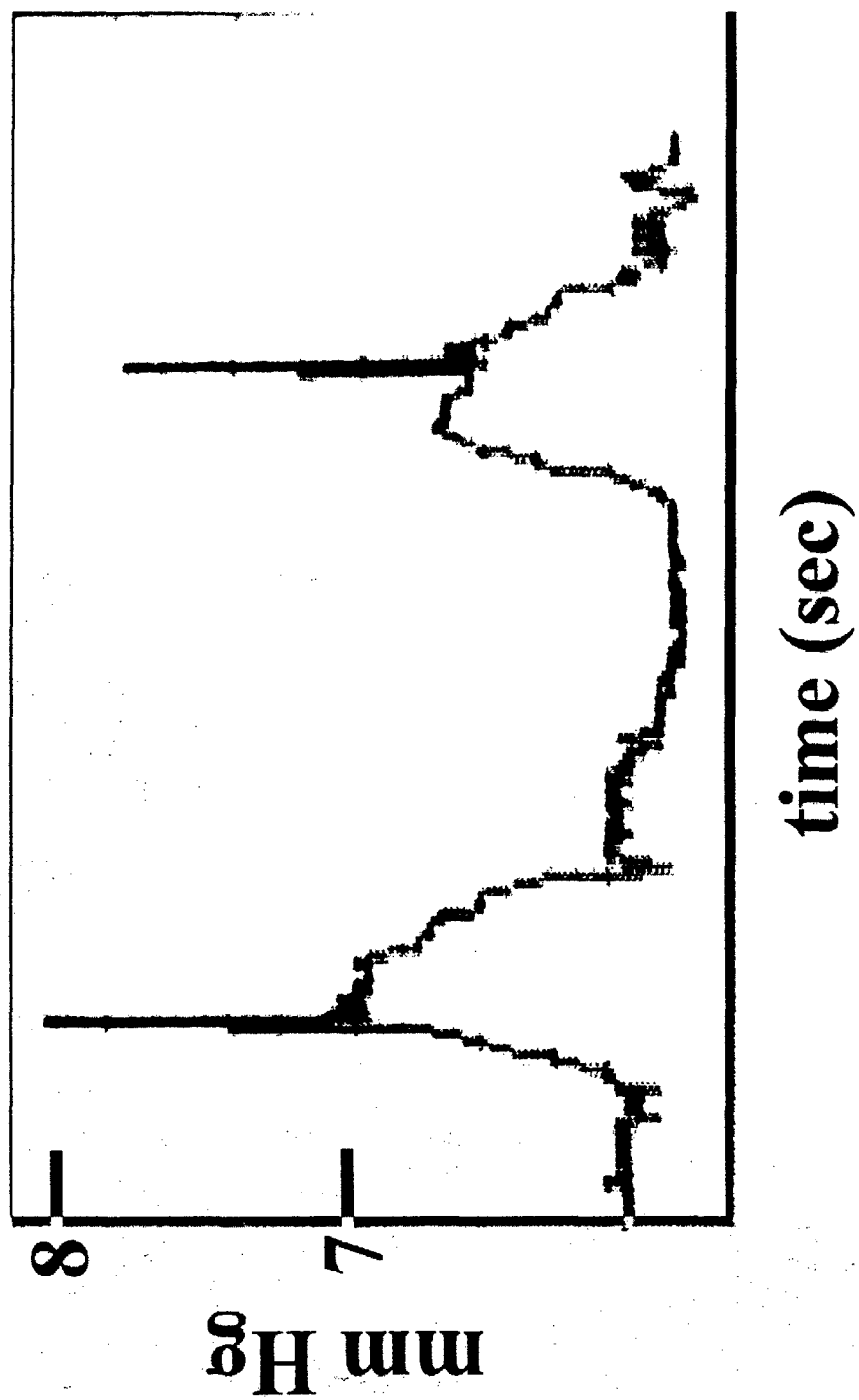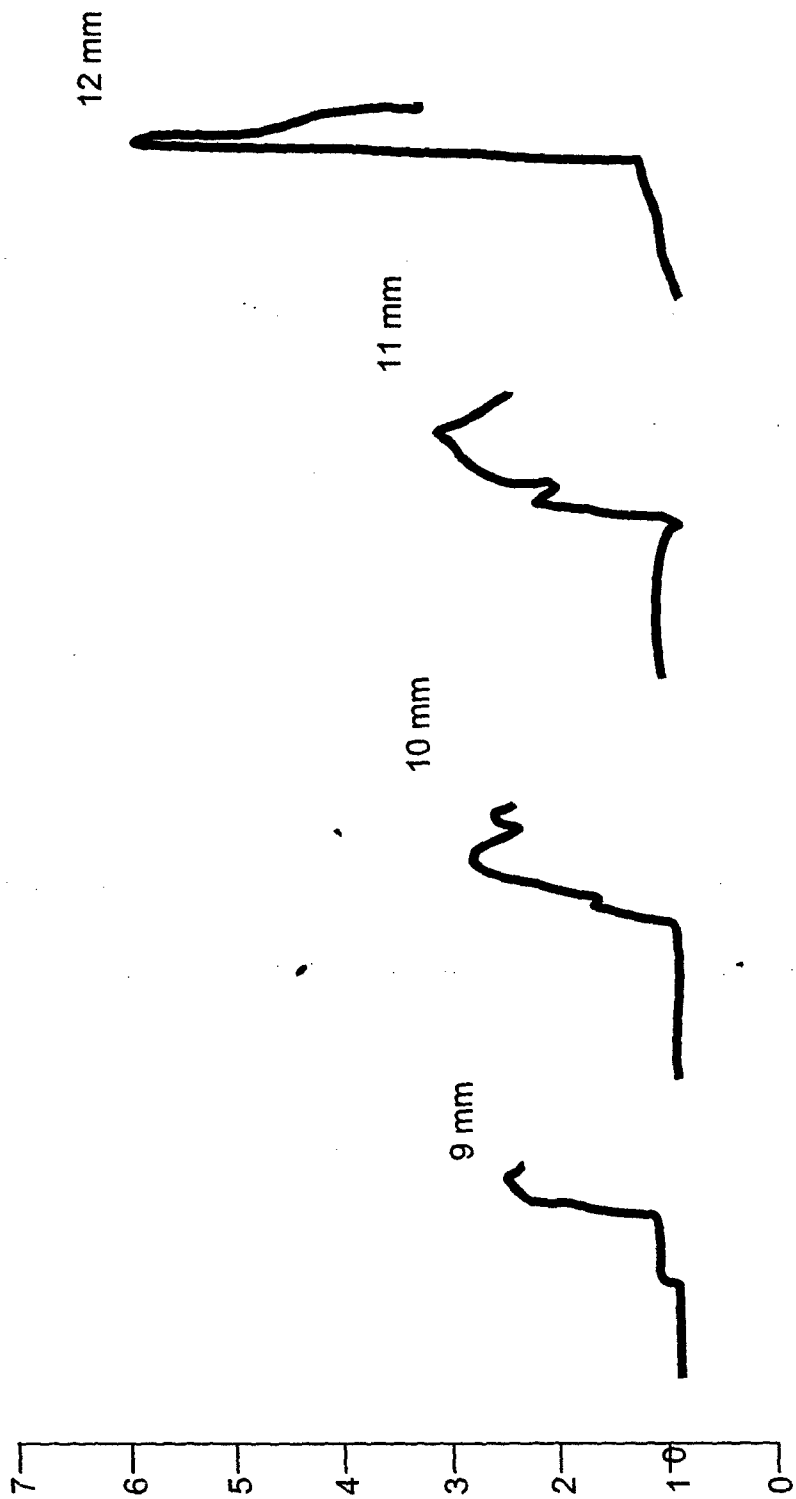
Normal 9 mm 10 mm 11 mm
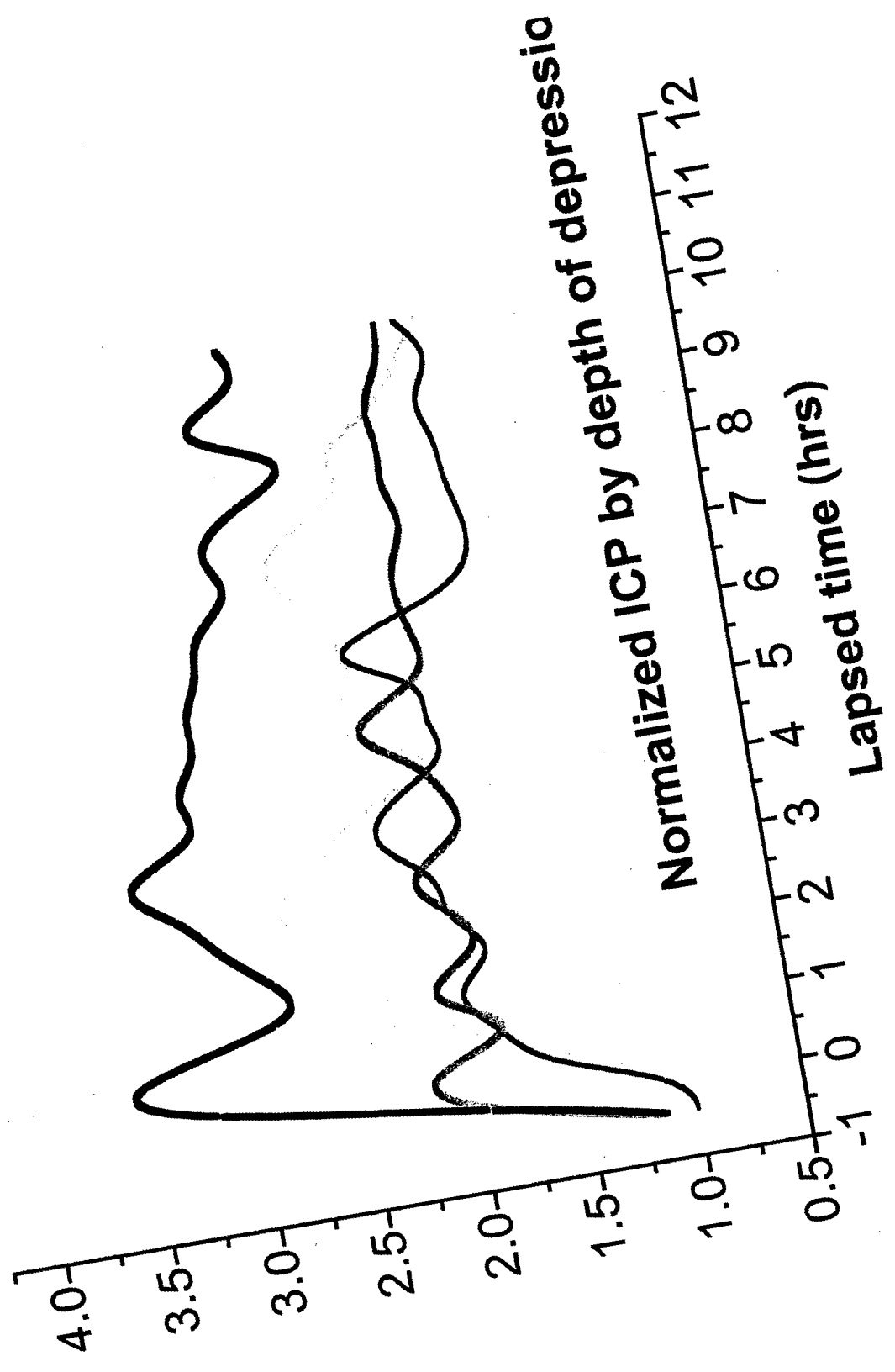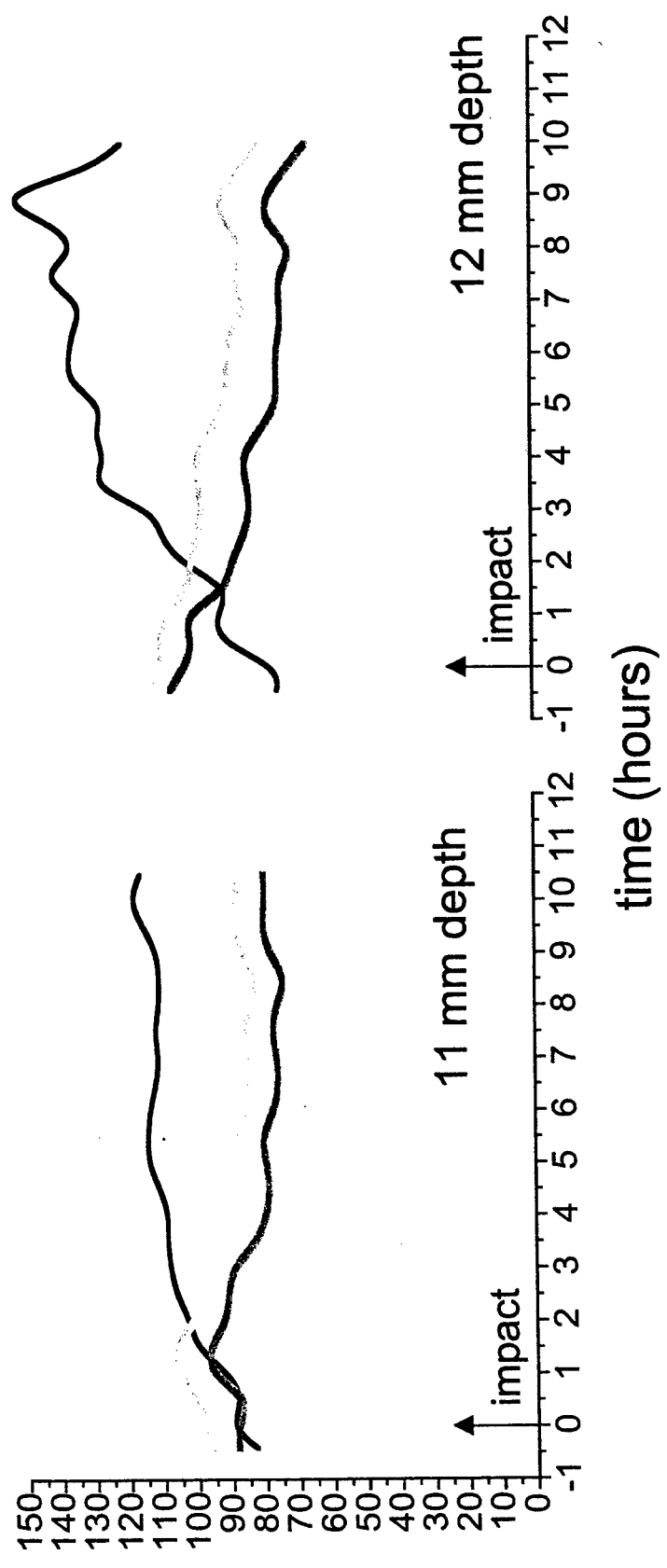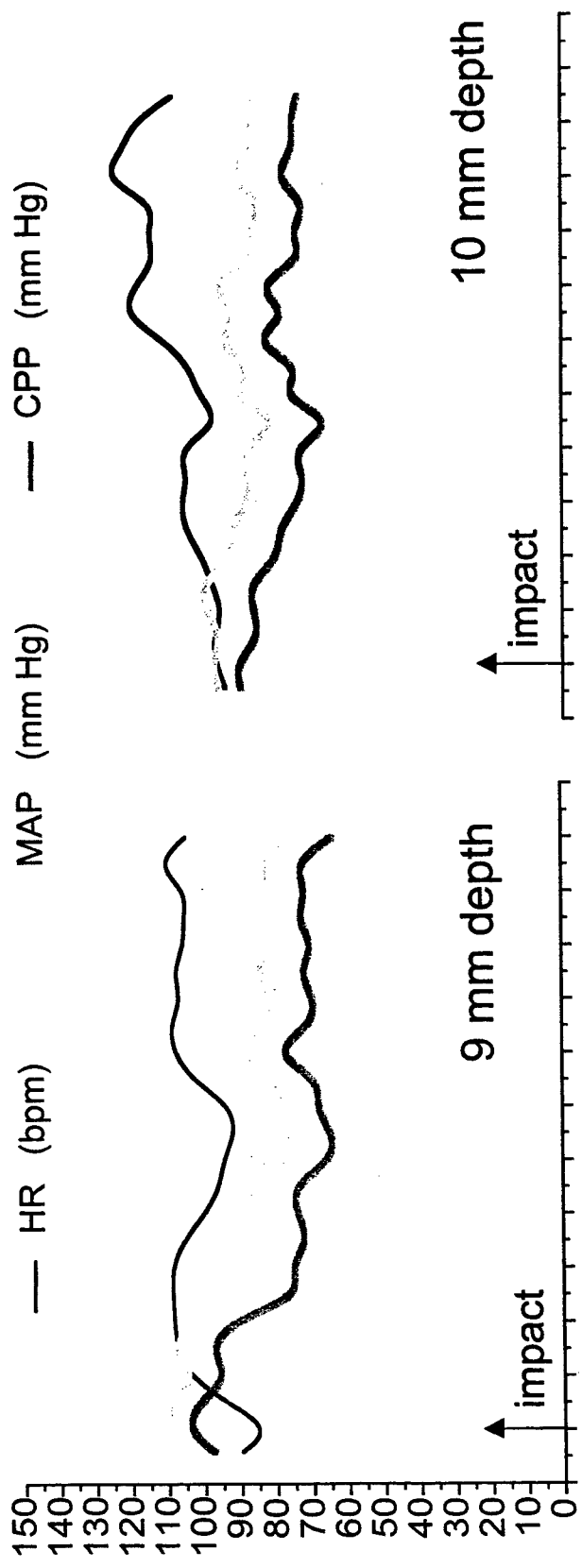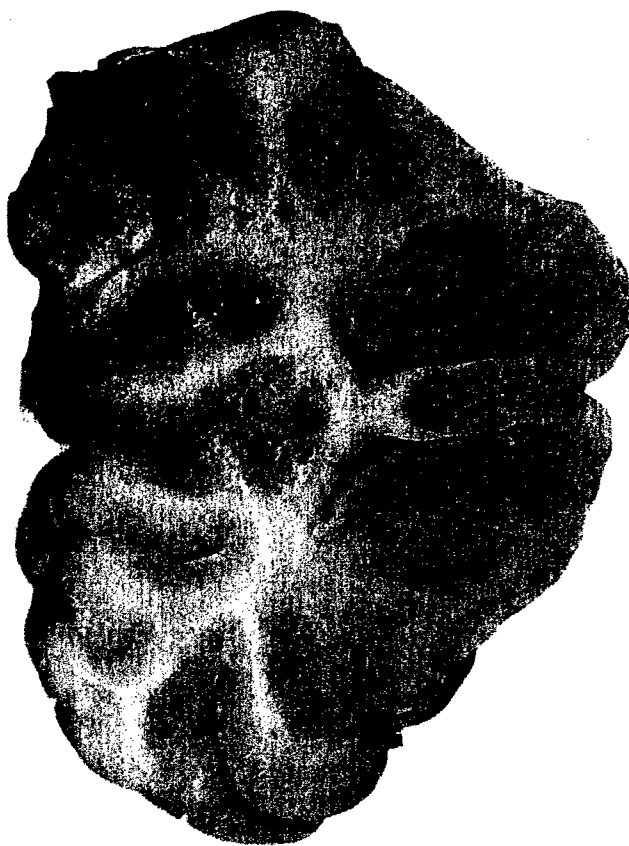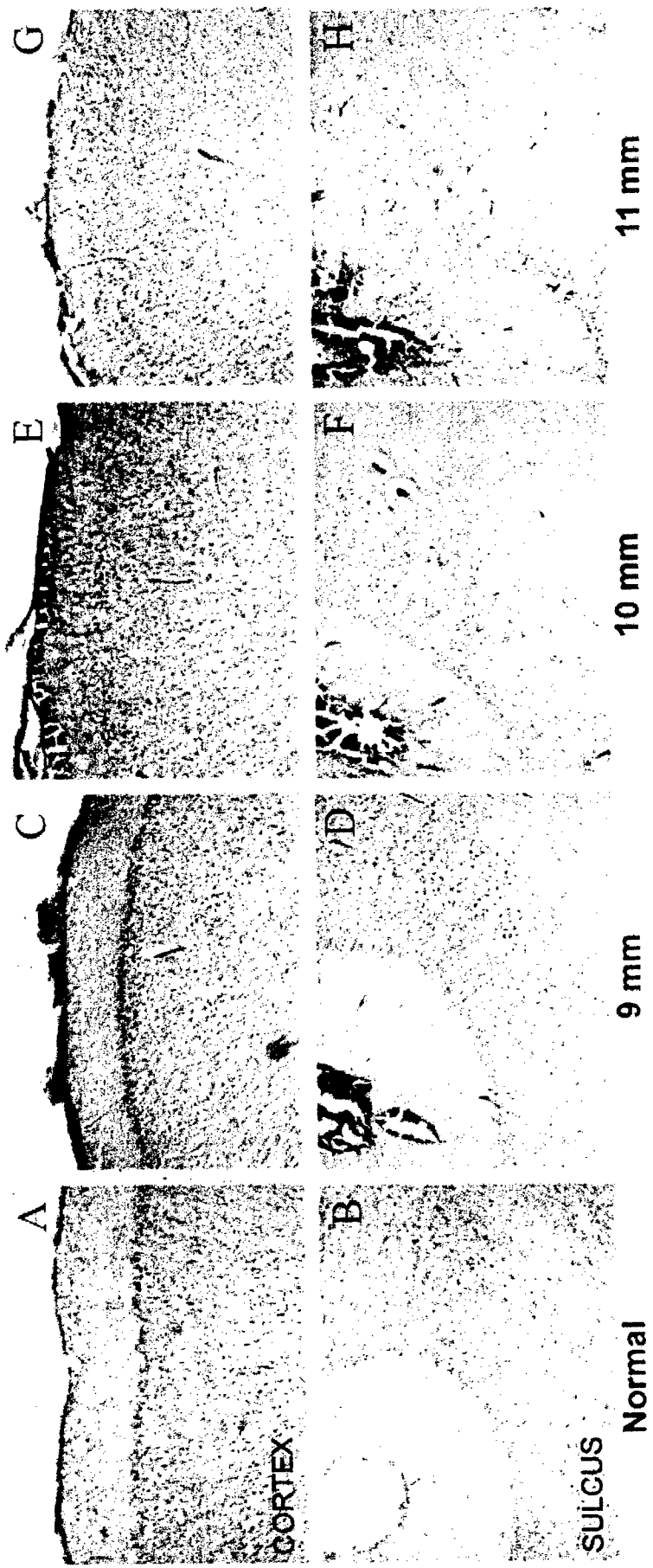
	uninjured	injured
MAP-2	A	B
MAP-2	C	D
NeuN	E	F
Fluoro-Jade B	G	H
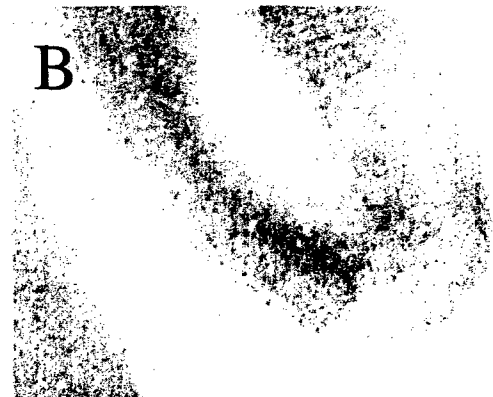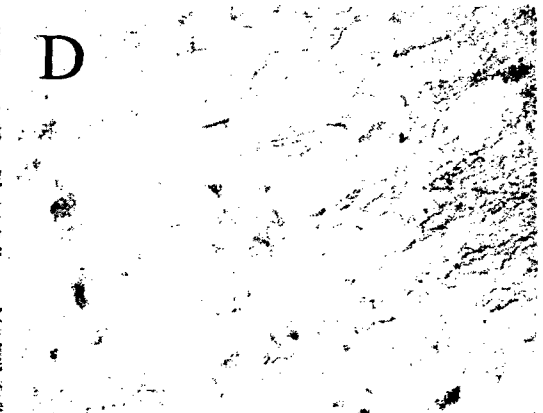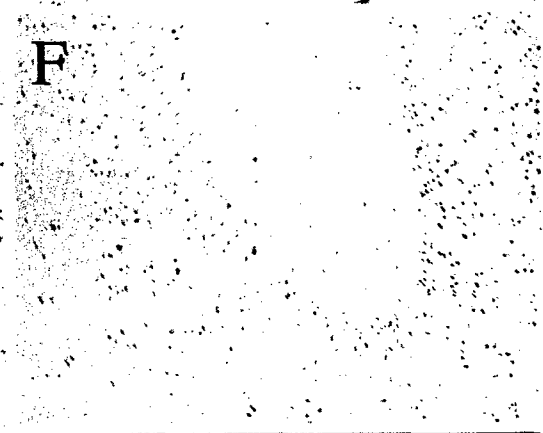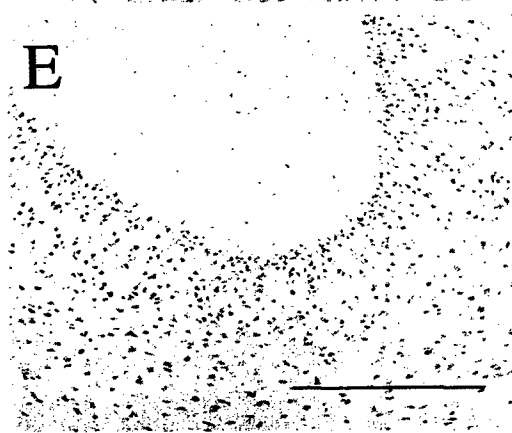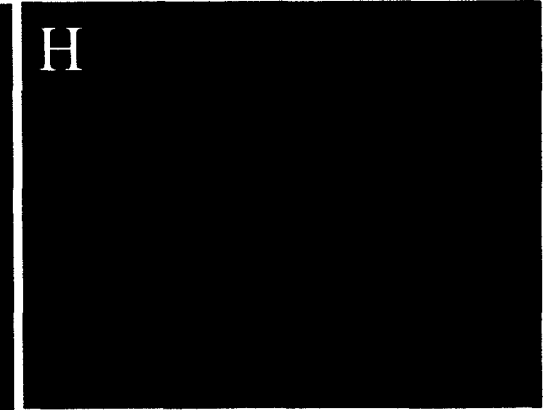
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14. ABSTRACT
A Controlled cortical impact (CCI) device was used to generate a focal lesion in 23 anesthetized male Yorkshire swine. In 10 swine CCI parameters of velocity and dwell time were varied to achieve a consistent injury (3.5 m/sec, 400 m/sec, respectively). In 13 swine depth of depression was varied from 9 to 12 mm. Physiological data including heart rate (HR), mean arterial blood pressure (MAP), intracranial pressure (ICP), and cerebral perfusion pressure (CPP) were collected for 10 hours after injury. Following injury, ICP and HR increased above baseline values in all swine with a more pronounced elevation in animals impacted to a depth of depression of 12 mm. An 11 mm depth of depression was found to most closely mimic pathological features of human TBI with edema, infiltration of inflammatory cells, pericapillary hemorrhage, and petechial hemorrhages in the white matter. Injury to a depth of depression of 12 mm resulted in cortical laceration obscuring these features. Immunohistological staining with Neu-N, MAP-2, and Fluoro Jade B revealed evidence of degenerating neurons, axonal disruption, and impending cell death. These results indicate that the swine model of CCI results in a defined and reproducible injury with pathological features similar to human TBI

15. SUBJECT TERMS
Traumatic brain injury; Head injury; Neuromonitoring, Controlled cortical impact; Swine

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