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# Modification of the Cortical Impact Model To Produce Axonal Injury in the Rat Cerebral Cortex

D.F. MEANEY,[1] D.T. ROSS,[2] B.A. WINKELSTEIN,[1] J. BRASKO,[2] D. GOLDSTEIN,[1] L.B. BILSTON,[1] L.E. THIBAULT,[1] and T.A. GENNARELLI[2]



## ABSTRACT

**Diffuse axonal injury (DAI) is a form of brain injury that is characterized by morphologic changes to axons throughout the brain and brainstem. Previous biomechanical studies have shown that primary axonal dysfunction, ranging from minor electrophysiologic disturbances to immediate axotomy, can be related to the rate and level of axonal deformation. Some existing rodent head injury models display varying degrees of axonal injury in the forebrain and brainstem, but the extent of axonal damage in the forebrain has been limited to the contused hemisphere. This study examined whether opening the dura mater over the contralateral hemisphere could direct mechanical deformation across the sagittal midline and produce levels of strain sufficient to cause a more widespread, bilateral forebrain axonal injury following cortical impact. Intracranial deformation patterns produced by this modified cortical impact technique were examined using surrogate skull-brain models. Modeling results revealed that the presence of a contralateral craniotomy significantly reduced surrogate tissue herniation through the foramen magnum, allowed surrogate tissue movement across the sagittal midline, and resulted in an appreciable increase in the shear strain in the contralateral cortex during the impact. To evaluate the injury pattern produced using this novel technique, rat brains were subjected to rigid indentor impact injury of their left somatosensory motor cortex (1.5 mm indentation, 4.5–4.9 m/sec velocity, and 22 msec dwell time) and examined after a 2–7 day survival period. Neurofilament immunohistochemistry revealed numerous axonal retraction balls in the subcortical white matter and overlying deep cortical layers in the right hemisphere beneath the contralateral craniotomy. Retraction balls were not seen at these positions in normals, sham controls, or animals that received cortical impact without contralateral craniotomy and dural opening. The results from these physical modeling and animal experiments indicate that opening of the contralateral dura mater permits translation of sufficient mechanical deformation across the midline to produce a more widespread pattern of axonal injury in the forebrain, a pattern that is distinct from those produced by existing fluid percussion and cortical impact techniques.**

## INTRODUCTION

DIFFUSE BRAIN INJURY ASSOCIATED WITH PROLONGED COMA without intracranial mass lesion is the most common form of brain injury in severely head injured patients (44%) and displays the second highest mortality rate among all types of head injury (Gennarelli et al., 1982a). Diffuse axonal injury (DAI) is the primary

[1]Department of Bioengineering and [2]Division of Neurosurgery, University of Pennsylvania Head Injury Center, Philadelphia, Pennsylvania.

pathological feature of diffuse brain injury and is characterized by morphologic changes to axons throughout the brain and brainstem. Experimentally, a spectrum of brain injuries including mild, moderate, and severe DAI has been produced in subhuman primates using a noncontact, angular acceleration of the animal head (Gennarelli et al., 1982b). Biomechanical modeling studies of these experiments revealed that axonal injury occurring in specific regions of the brain could be related to the magnitude of strain occurring in these regions (Margulies et al., 1990). Because of biomechanical scaling constraints, however, this angular acceleration model of traumatic brain injury (TBI) has not been extended for use in small animal species, such as the rat, cat, or ferret. Rather, the most common method to produce TBI in small animals is direct brain deformation, where local mechanical insults to the exposed cortical surface rapidly deform the brain and produce TBI.

The most common techniques to produce brain injury in small animals include central (midline) or lateral fluid percussion, weight drop, and cortical impact with a rigid indentor. A detailed description and review of the histologic findings derived from these models can be found elsewhere (Gennarelli and Thibault, 1984; Lighthall et al., 1989). Briefly, severe to moderate central fluid percussion injuries have been found to produce lesions in the lower brainstem region (Dixon et al., 1988; Shima and Marmarou, 1991) as a result of the high strains appearing in this area during percussion loading (Thibault et al., 1993). Moreover, axonal injury in central fluid percussion appears primarily surrounding the site of impact and in the brainstem (Povlishock and Becker, 1985). In contrast, lower brainstem damage is not as extensive for similar loading conditions using the lateral fluid percussion technique in the rat (McIntosh et al., 1987). Rather, lateral fluid percussion in the rat produces an intriguing pattern of axonal damage that begins at the vicinity of the impact site and extends into the ipsilateral internal capsule and fimbria.

In comparison, the weight drop technique (Feeney et al., 1981) has been found to produce a contusion surrounding the impact site and effect diffuse changes in cerebral metabolism. Common pathologic features of the technique include hemorrhage and long-term cortical necrosis at the impact site. Although the depth and extent of the contusional injury can be controlled by varying the weight and velocity at which the impounder strikes the cortical surface, the biomechanics of this technique have not been fully investigated.

In its present form, the cortical impact method developed for use in the ferret and rat (Dixon et al., 1991; Lighthall, 1988) is similar to the central fluid percussion technique because cortical impact produces contusion and associated axonal injury at the indentation site for high level injury. Additionally, lower brainstem damage has been noted for severe levels of cortical impact in the ferret (Lighthall, 1988). One primary advantage of the cortical impact technique is the precise control of the indentor and rapid removal (< 100 msec) of the indentor after impact, therefore eliminating the problem of residual fluid volume that can occur with fluid percussion. However, the biomechanical aspects of cortical impact injury, in particular the manner in which the pattern of intracranial deformation relates to the distribution and type of brain injuries produced, have not been studied extensively.

In this report, we develop a new experimental model of TBI that uses cortical impact to produce a more diffuse distribution of axonal injury in comparison with other small animal models of TBI. Specifically, since the corpus callosum and subcortical white matter are frequent foci of axonal damage in severe DAI (Adams et al., 1982; Grcevic and Jacob, 1965; Strich, 1956), we sought to determine whether we could direct strain across the sagittal midline in a rodent cortical impact model to selectively damage similar anatomic regions in the rat. To accomplish this selective damage, we removed contralateral bone and underlying dura mater before cortical impact. A two-part study of this modification to the rigid indentor cortical impact model is described in this investigation. First, physical modeling studies were performed with surrogate models of brain-skull structure to directly measure the effects of a contralateral craniotomy on surrogate tissue strain levels in the contralateral cortex. Second, the biomechanical parameters used in this surrogate model analysis were scaled to produce similar types of deformation in the smaller rat brain, and a series of animals were injured using the modified impact technique to evaluate the ability to produce forebrain axonal injury in vivo.

## MATERIALS AND METHODS

### *Injury Device*

To study the biomechanical aspects of the modified cortical impact technique, a pneumatic impactor device similar to the device developed by General Motors Research Laboratories (Lighthall, 1988) was constructed

(Fig. 1). Briefly, this apparatus consists of a pneumatic cylinder (Bimba Co., Monee, IL) coupled to two independently controlled solenoid valves. A gas pressure source was attached to the solenoid valves, and the actuation of the valves was controlled by an electrical timing circuit. The timing circuit was designed to permit the duration of impact to vary across a wide range (25 msec–3 sec). For these studies, impact duration was maintained within a range of 22–25 msec.

The tip of the pneumatic cylinder shaft was fitted with an indentation tip [diameter 12.5 mm (physical model), diameter 3 mm (animal studies)], and the cylinder body was adapted to a Kopf stereotaxic X-Y manipulator (David I Kopf, Tujunga, CA). The indentor was lowered onto the surface of the surrogate gel or brain tissue, retracted into its original position, and then adjusted to deliver an indentation of a given depth (±0.2 mm). Velocity of the indentation was controlled by adjusting the pressure supplied to the pneumatic cylinder and was within the range of 2.8–3.0 m/sec for both the physical model and animal experiments. For each test, the indentation displacement was measured using a linear variable differential transformer (LVDT) transducer (Trans-Tek Inc, Ellington, CT).



### *Physical Model Construction and Analysis*

The techniques used in constructing the physical model were as previously described (Margulies et al., 1990; Meaney and Thibault, 1990; Thibault et al., 1993). Briefly, a coronal section model was constructed by cutting a cat skull (Carolina Biological Supply, Burlington, NC) coronally, 3 mm anterior to the mandibular fossa, and coating the interior of the skull with white enamel (Testors, Inc.) to enhance contrast for high speed cinematography. The surrogate model was fitted with a surrogate cervical spinal column machined to insert into the foramen magnum of each skull. Craniotomies located at the vertex of the skull and along each lateral surface (90 degrees from the sagittal midplane) were incorporated into the model. Depending on the tests conducted, these craniotomies were either left open or capped with a rigid epoxy to simulate the rigid skull. An illustration of the completed physical model used to investigate lateral cortical impact is shown in Figure 2.

The interior of the model was filled with a silicone gel material (Sylgard gel, Dow Corning, Midland, MI), which served as a surrogate for brain tissue. This material was used because it (1) exhibits mechanical properties similar to those of brain tissue (Blum et al., 1985), (2) is optically transparent, and (3) is self-adherent so that a series of layers can be cast to form a continuous material that is void of mechanical discontinuities at the interfaces. The silicone gel material was cast in two layers, and an orthogonal grid with 2.5 mm spacing was placed in a coronal plane 4 mm below the surface face. The model was subjected to impact with peak indentation at 15% of the brain width, and the velocity was fixed at 4.5 m/sec.

Deformation of the grid pattern in the surrogate brain tissue was filmed using a high speed camera (HYCAM II, Redlake Industries, Irvine, CA) operating at a rate of 6500 frames per second. A time series showing the deformation occurring in response to the impact for each experiment was produced from 8 × 10 inch photographs of frames from the high speed film. The location of the grid in each photograph was recorded using a digitizing

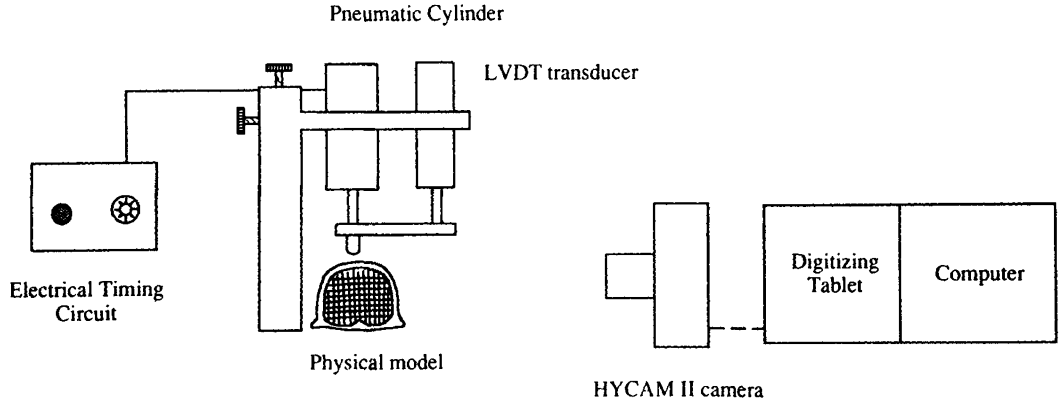


**FIG. 1.** Schematic of cortical impact apparatus, consisting of a pneumatic cylinder coupled to two independently controlled solenoid valves. Pressure supplied to the solenoid valves permitted varying the impact velocity, and a timing circuit controlling the solenoid valve operation allowed the indentation duration to vary across a wide range (25 msec–3 sec).

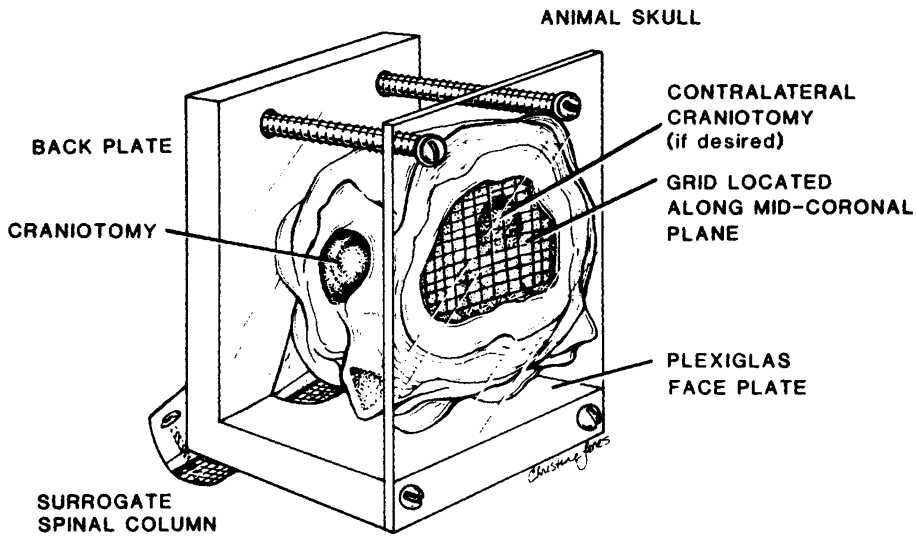


**FIG. 2.** Illustration of physical model used to investigate cortical impact. A cat skull was cut coronally, fitted with a surrogate cervical spinal column, and filled with a silicone gel material intended to simulate the mechanical properties of brain tissue. Distortion of a grid located in the midcoronal plane was used to measure the strain caused by lateral impact to the cortex.

tablet (Summagraphics Inc., Seymour, CT) and stored on computer (Dell Computer Corp., Austin, TX). For surrogate brain regions of interest, the digitized images were recalled to calculate strain. Shear strain ($\gamma$) (Fung, 1965) produced in the surrogate tissue in response to cortical impact was determined by analyzing the maximum change in angle formed by line segments extending from a selected node in the grid pattern compared to the underformed state. Displacement ($\delta$) was calculated as the movement of a given nodal point from its original, preimpact position. For the measurements presented in this study, shear strain and displacement were determined from the deformation patterns of the physical model at sites both local to and remote from the impact location (Fig. 3). These regions were studied for two conditions: (1) ipsilateral craniotomy only and (2) bilateral craniotomies.

### *Animal Preparation and Injury*

Based on the results of the physical model studies, the impact parameters were scaled for the smaller rat brain, and the angle of impact was adjusted for impact to the somatosensory motor cortex. Scaling was accomplished by maintaining the same fractional compression, defined as the indentation depth divided by the dor-

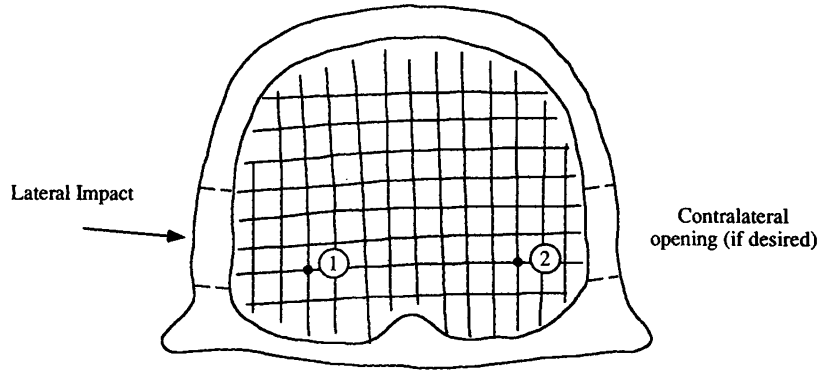


**FIG. 3.** Schematic of areas examined in the physical model tests, intended to encompass regions both near (**1**) and remote (**2**) from the impact site.

sal–ventral brain distance, for both the physical model and animal tests. Impact velocity was held constant for both test series.

For the animal studies, adult male Long-Evans rats (320–450 g) were anesthetized and mounted in a stereotaxic frame. Bilateral parietal craniotomies (4mm diameter) were made centered at 1.8 mm behind bregma and 4 mm lateral to midline, over the hindlimb region of the somatosensory motor cortex. The dura mater overlying the right cortex was reflected immediately before indentation. Care was taken to avoid producing inadvertent cortical contusion, laceration, or subdural hematoma before impact. Cases where confounding cortical injury before impact had occurred were rare and are excluded from this analysis. The left cortex was indented 1.5 mm at a 23-degree angle (normal to the dural surface at the center of the impact site) with a velocity of 4.5–4.9 m/sec and a 22–25 msec dwell time. Each animal received a single impact. Any bleeding after impact was controlled before suturing the skin incision and before the animal fully emerged from anesthesia. After emergence from anesthesia, the animals were returned to the animal colony. Two to seven days later, animals were killed by lethal injection of Nembutal and then perfused transcardially. Animals were exsanguinated by perfusion with a 0.1M phosphate-buffered saline (PBS) solution containing 1 unit/mL of heparin, fixed by perfusion with a 4% paraformaldehyde, 0.1% glutaraldehyde solution in PBS (pH 7.4), and postfixed with a 10% sucrose PBS solution. Brains were stored in 30% sucrose saline. Frozen sections were cut at 40 μm sections in the coronal or sagittal plane, and six parallel sets of serial sections were saved.

Individual sets were either stained with cresyl violet or reacted for immunohistochemistry using antiserum to glial fibrillary acidic protein (GFAP) or SMI-32, a mouse monoclonal antibody (mAB) to nonphosphorylated epitopes on heavy and medium neurofilament subunits. The SMI-32 antiserum was obtained from Sternberger-Meyer Monoclonals, and the polyclonal rabbit anti-GFAP antiserum was a gift from Dr. Larry Eng. The results from cortical impact cases with contralateral dural opening ($n = 28$ animals) were compared with those from sham controls ($n = 3$ animals or $n = 6$ cortical hemispheres), which received bilateral dural opening without cortical impact, with those receiving cortical impact without contralateral craniotomy ($n = 6$ animals), and with those receiving cortical impact with contralateral craniotomy in which the contralateral dura was left intact ($n = 6$ animals).

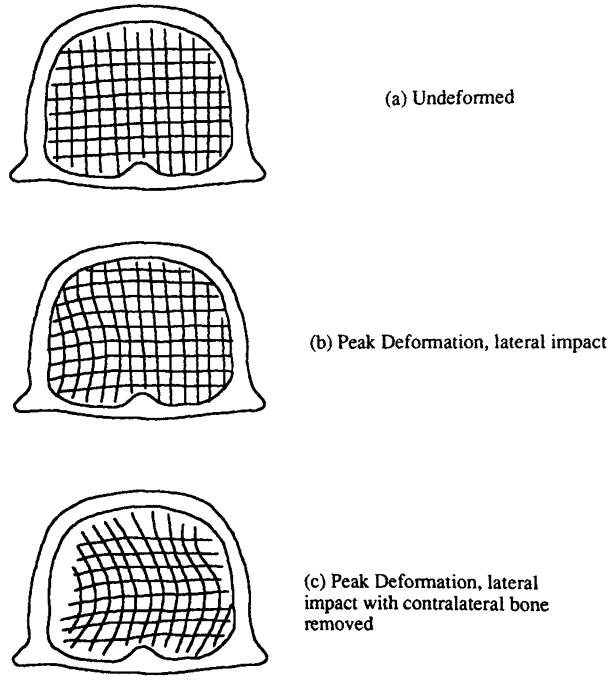


**FIG. 4.** Digitized reconstructed images of coronal plane grid deformation caused by lateral impact for **(b)** unilateral and **(c)** bilateral craniotomy configurations. Note the pronounced displacement of the grid pattern across the sagittal midplane when a contralateral opening is used **(c)**.

## RESULTS

### *Biomechanical Studies*

Qualitatively, digitized reconstructed images of the physical model grid deformation pattern illustrate the effect of incorporating a contralateral opening before impact (Fig. 4). By providing an alternative opening for surrogate tissue, material preferentially moves across the sagittal midline and extrudes through the contralateral opening. Quantitatively, an appreciable amount of tissue displacement occurs at the impact site even when contralateral bone is left intact during cortical impact, but little displacement is evident at the position of the contralateral cerebral cortex (Fig. 5A). Similarly, in the absence of a contralateral craniotomy, shear strains are confined to an area local to the indentation site (Fig. 6A).

Conversely, incorporating a contralateral craniotomy significantly increased the displacement in the contralateral cortex (Fig. 5B). Because of the positioning of the craniotomies, surrogate tissue moved primarily laterally to extrude through the contralateral opening. The initial overshoot of surrogate tissue displacement is a result of the inertial characteristics of the silicone gel and may provide a partial explanation of the velocity-dependent injury patterns observed for this model. As a result, shear strains appeared in both the ipsilateral and contralateral cortex, thereby creating a more widespread deformation field (Fig. 6B).

### *In Vivo Neuropathologic Studies*

At the impact site, a small subdural hematoma invariably was produced immediately following injury. By 7 days, however, the hematoma had resolved and was not apparent at the time of death. Within cortical white



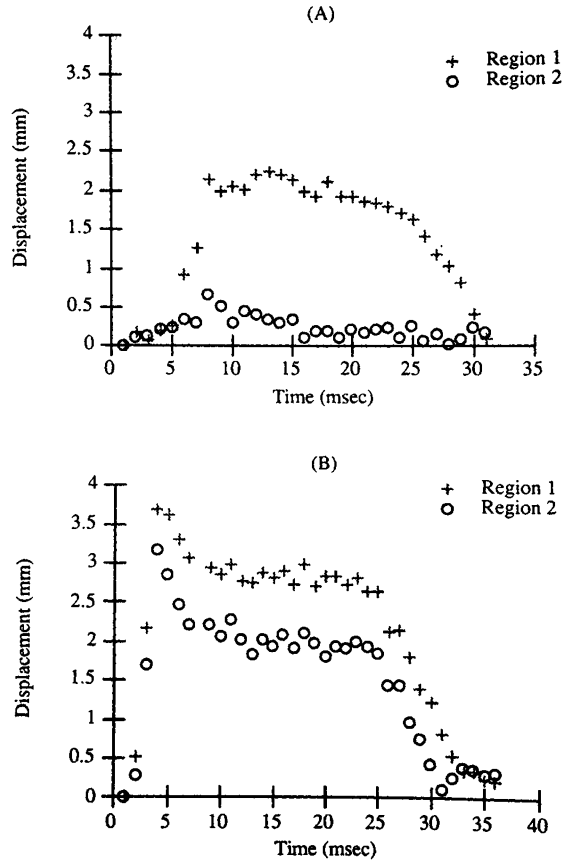


**FIG. 5.** Peak displacement (δ) of surrogate brain tissue caused by cortical impact for **(A)** unilateral and **(B)** bilateral craniotomy configurations. Using a contralateral craniotomy moves surrogate tissue across the sagittal midline and reduces the movement of surrogate material through the foramen magnum.

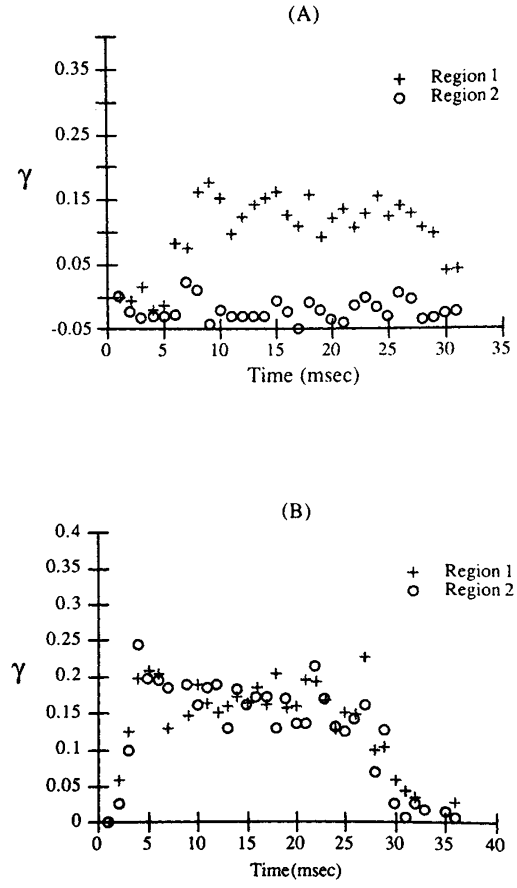


**FIG. 6.** Peak shear strain ($\gamma$) measured during impact loading period for **(A)** unilateral and **(B)** bilateral craniotomy configurations. A single craniotomy configuration creates strains confined to the impact site, whereas introducing a contralateral craniotomy creates a more widespread deformation field.

matter beneath the impact site, numerous axonal retraction balls were positively labeled with the SMI-32 neurofilament antibody. In Nissl-stained sections, a minor lesion was observed in the superficial layers (II–IV) of the impacted cortex (Fig. 7B). This lesion consisted of a loss of neurons in several cortical layers, as well as a pronounced glial proliferation (Fig. 8A). The axonal damage observed in the impacted cortex was not dependent on removal of contralateral bone, since no differences were observed when comparing brains injured using a unilateral craniotomy to those injured using a bilateral craniotomy configuration.

In the contralateral cerebral cortex, however, axonal damage was dependent on removal of contralateral dura mater before impact. A set of experiments ($n = 6$ animals) was conducted in which contralateral bone was removed but the underlying dura mater was left intact. The pattern of axonal damage was restricted to the impacted hemisphere and was similar in nature to results from experiments in which only one craniotomy was used.

In cases where contralateral dura mater was removed, the contralateral cortex was transiently extruded 1–2 mm out the craniotomy after impact. Tissue herniation was maintained at a level about 1–1.5 mm above the level of the previous dural surface by subsequent parenchymal swelling. Cortical swelling was sometimes accompanied by bleeding at the edges of the craniotomy hole or blanching of cortical vessels and an evident extravasation of the blood from the vessels into the cortical parenchyma. Immunohistochemistry (SMI-32) revealed the presence of axonal retraction balls at the white matter–gray matter junction and also in the deeper white matter (Fig. 9). In Nissl-stained sections from these animals killed 2–7 days after cortical impact, the contralateral somatosensory cortex area beneath the contralateral craniotomy was characterized by the hypertrophy of neurons in layers II and III of the cortex (Fig. 8C). In some cases, a microglial reaction and the pres-

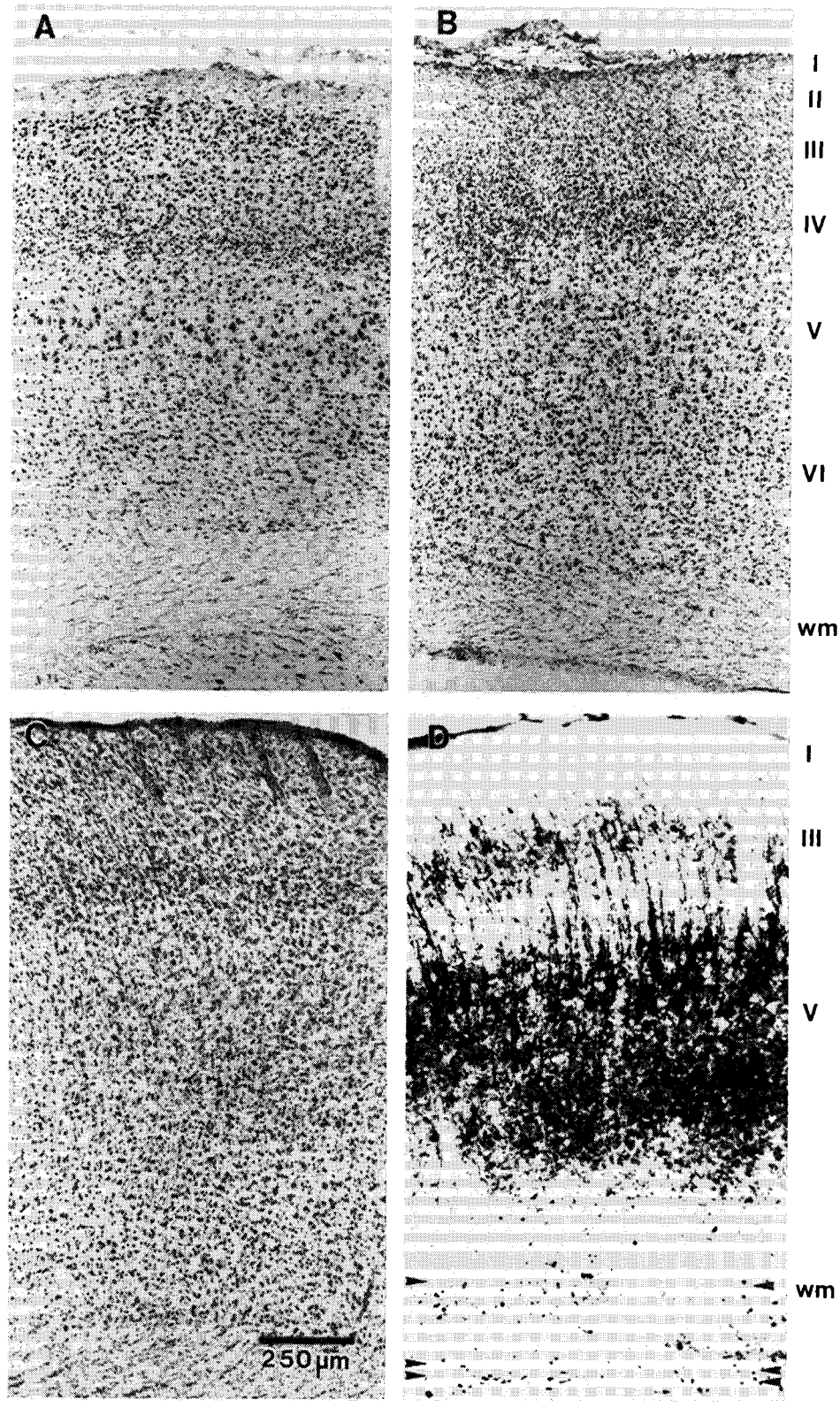


**Fig. 7.** Somatosensory cortex 6 days following impact with rigid indentor. **A.** Normal cortex. Cresyl violet stain. **B.** Impacted cortex. Cresyl violet stain. **C.** Cortex beneath contralateral craniotomy. Cresyl violet stain. Note the minor lesion in the superficial layers (II–IV) of the impacted cortex **(B)** and the marked swelling of the contralateral cortex **(C). D.** Adjacent section to C labeled with a neurofilament antibody. Note the presence of retraction balls at the white matter–gray matter junction (single arrow) and in the deeper cortical white matter (double arrows).

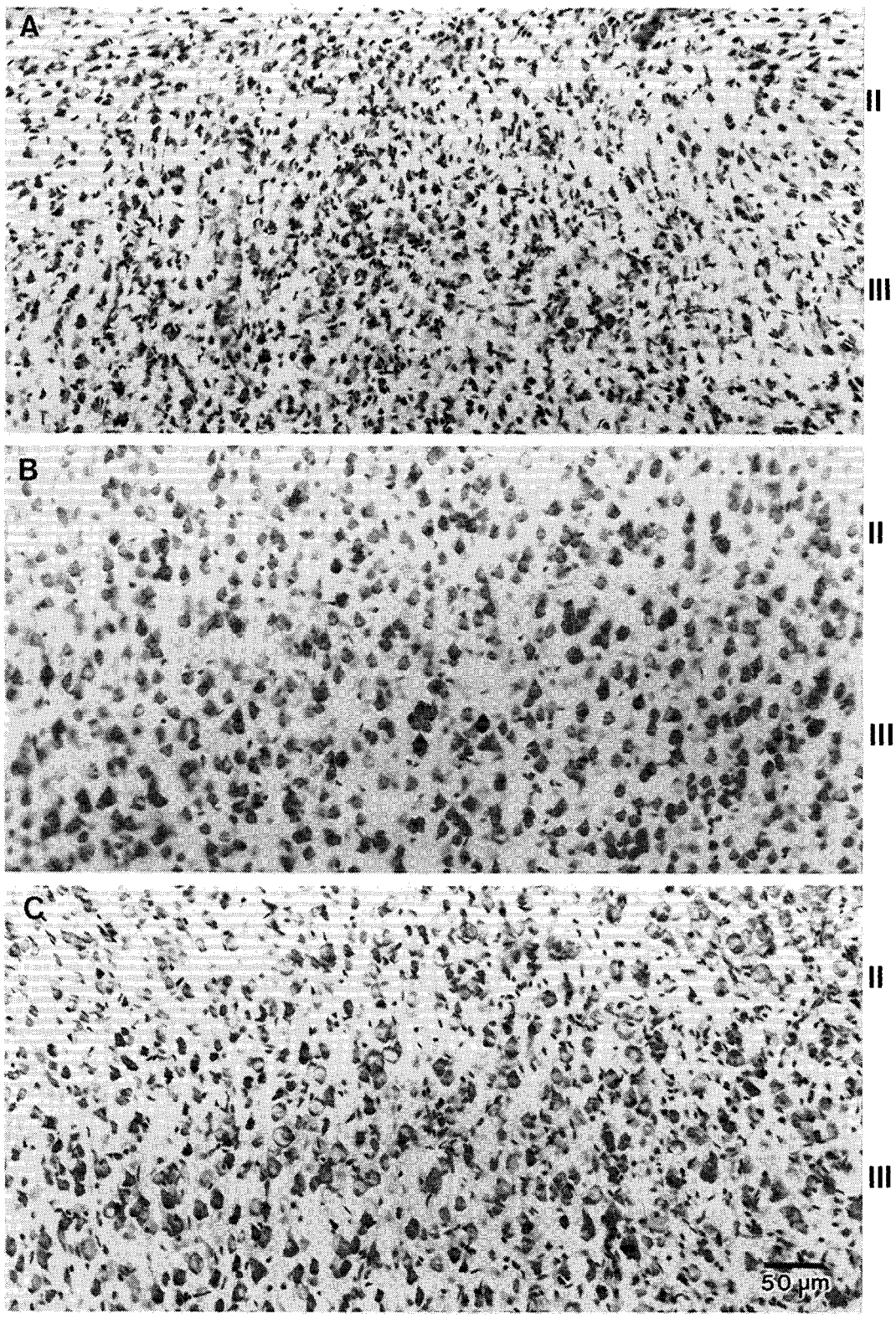


**FIG. 8.** Detail of lesion in the superficial cortex, cresyl violet stain. **A.** Contralateral cortex beneath contralateral craniotomy 7 days after cortical impact. The loss of neurons from layers II and III and the proliferation of small glial cells are evident. **B.** Layers II and III of the normal somatosensory cortex. **C.** Contralateral cortex below dural opening 7 days after cortical impact. A marked hypertrophy of neurons in layers II and III is visible.

ence of hematogenous cells within the white matter indicated the presence of tears in the subcortical white matter beneath the contralateral craniotomy (Fig. 10B). Reactive astrocytes were present in the white matter and gray matter surrounding these tears and radiated out from the site of the tear (Fig. 10C). These tears were not continuous with lacerations on the cortical surface. Neurofilament immunohistochemistry revealed damage beneath the contralateral craniotomy and numerous large axonal retraction balls in the underlying cortical white matter (Fig. 9B). Retraction balls were not present in the corresponding regions of the white matter in cases where a contralateral craniotomy and dural opening had not been performed before impact, nor were retraction balls present in control cases. There was no evidence of subcortical axonal injury or subcortical neuronal degeneration in any of the injured brains. Finally, analysis of other brain regions (e.g., hippocampus, neocortex, mesencephalon, thalamus) revealed no evidence of axonal injury using the prescribed impact parameters.

## DISCUSSION

The results of these biomechanical and neuropathologic studies confirm the hypothesis that a novel distribution of axonal injury in the forebrain can be produced using cortical impact combined with a second craniotomy located contralateral to the impact site. Physical model studies indicate that cortical impact without contralateral craniotomy, the technique described by Lighthall (1988), produces deformation of material within the vicinity of the indentor tip and concomitant herniation of material through the foramen magnum. The magnitude of the localized deformation near the indentor tip suggests the development of levels of strain sufficient to cause neuronal damage. This prediction is borne out by the pattern of damage observed surrounding the impact site in this report and previous studies using the ferret and the rat (Dixon et al., 1991; Lighthall, 1988). In contrast, using a contralateral opening in the physical model substantially reduced herniation of material from the intracranial cavity and through the lower brainstem and increased the amount of deformation occurring in the forebrain region. As a result, axonal injury appeared in both the ipsilateral and contralateral hemispheres.

Several factors deserve consideration when interpreting the results contained in this study. First, the surrogate skull-brain physical model used for this study does not attempt to simulate the subtle differences in mechanical properties of distinct structures within the brain, such as the gray matter or subcortical white matter. Rather, the surrogate tissue used for construction of the model was selected to match the general mechanical properties of brain tissue as measured by indentation of the surface of the cerebral cortex (Blum et al., 1985). Therefore, the data generated from the physical modeling experiments in the present study represent an approximation of deformation caused by cortical impact in specific brain regions and may not predict the exact state of in vivo deformation that occurs in the inhomogeneous, anisotropic animal brain. As more detailed information on the constitutive properties of brain tissue becomes available, these features can be incorporated into future physical models. Second, because of the limits of grid resolution for analyzing deformation patterns in the physical model studies, a larger cat skull was used instead of a rat skull to obtain a more precise measure of deformation produced by cortical impact. Although an effort was made to scale the impact parameters accordingly by using intracranial volume changes, the precise scaling relationship to produce identical levels of deformation in brains of different sizes is not known. Nevertheless, the principal features of the deformation caused by cortical impact—strain in a region local to the indentor tip, herniation of surrogate brain tissue through the foramen magnum, and a more widespread pattern of strain appearing within the forebrain by incorporating a contralateral craniotomy–should not differ substantially between the two species studied in this report.

The appearance of axonal injury in the contralateral hemispheres of injured animals receiving a contralateral dural opening parallels the appearance of strain in the contralateral cortex measured in a physical model subjected to the same impact conditions. Axonal injury in the contralateral hemisphere, demonstrated using neurofilament immunohistochemistry, was evident primarily in the subcortical white matter and at the gray matter–white matter junction as terminal clubs on damaged cortical efferent or afferent axons. In cases of cortical impact without contralateral dural opening, axonal bulbs and swollen axonal segments were seen adjacent to the site of cortical impact, but similar axonal abnormalities were not evident either in the corpus callosum, subcortical white matter, or the gray matter of the contralateral cortex. Further, axonal abnormalities were not pre-

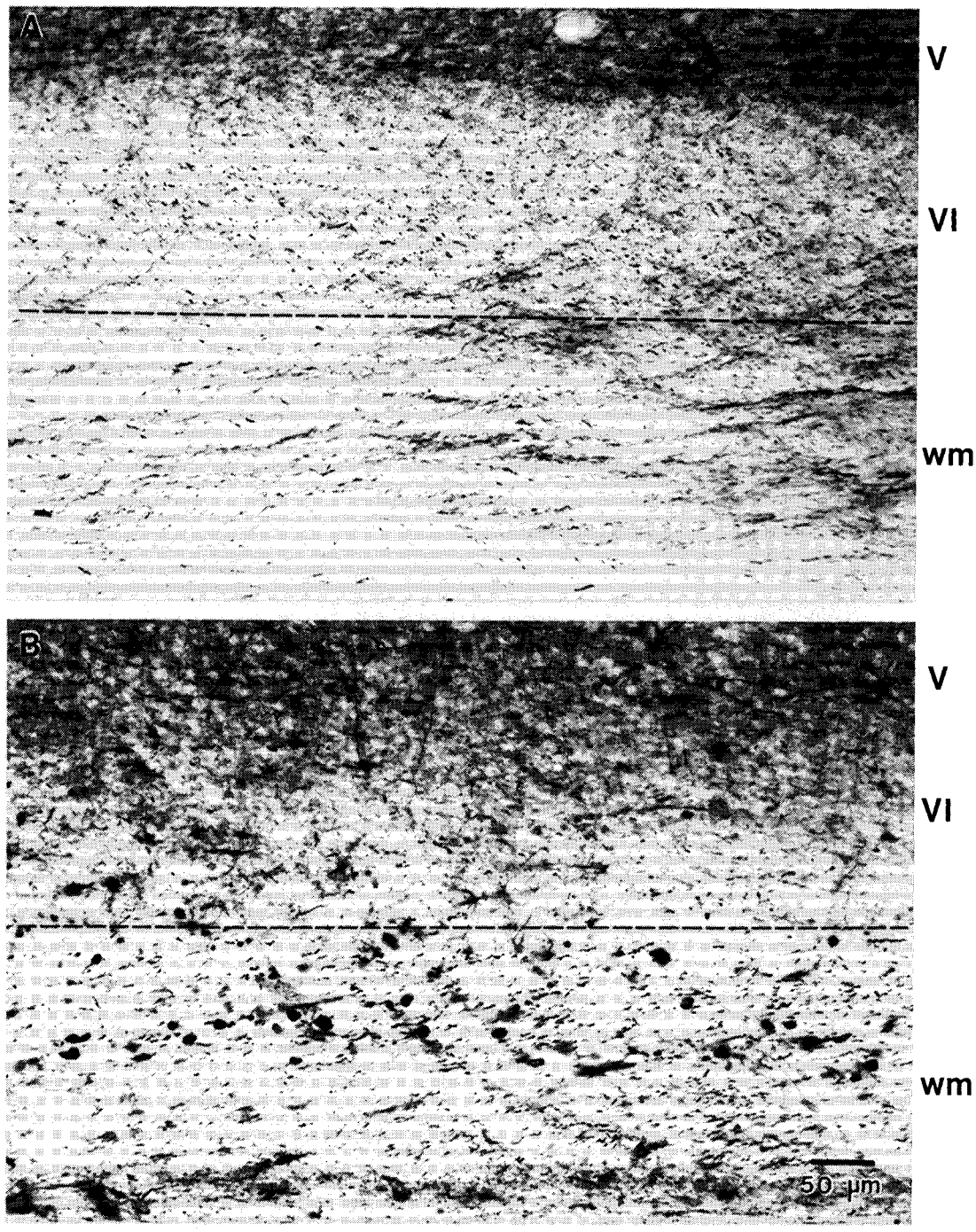


**FIG. 9.** Reaction of axons in the contralateral white matter to cortical impact. SMI-32 nonphosphorylated neurofilament immunohistochemistry. **A.** Normal subcortical white matter at the white matter–gray matter junction beneath the somatosensory cortex. **B.** Contralateral to the impacted cortex, beneath the dural opening, a decrease in the density of normal axons and a labeling of numerous swollen axonal segments and terminal clubs are evident within the white matter and at the white matter–gray matter junction.

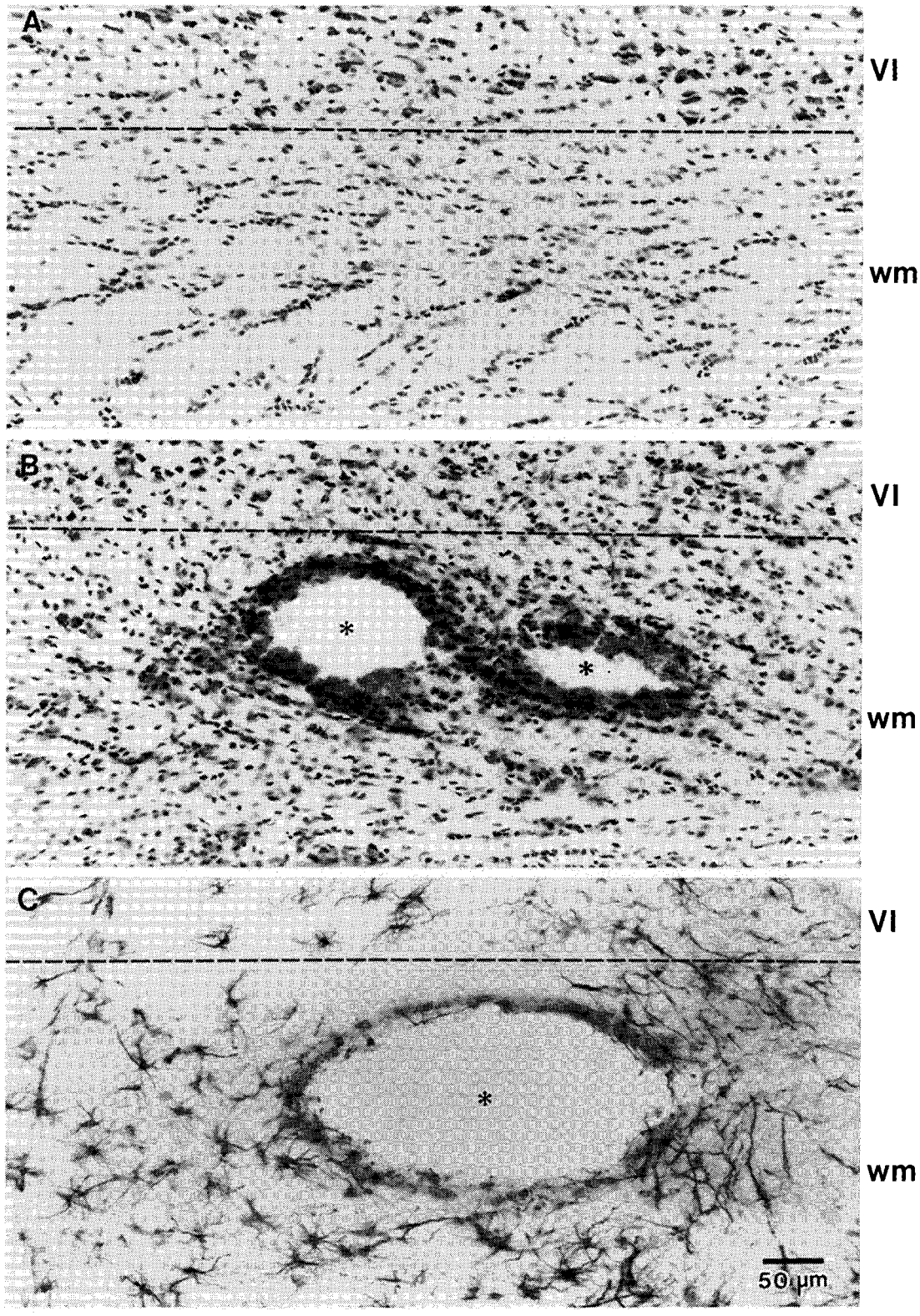


**FIG. 10.** Reaction of glia in the white matter beneath the nonimpacted contralateral cortex. **A.** Normal appearing rays of oligodendroglia in the white matter contralateral to impact. No contralateral craniotomy or dural opening performed. Cresyl violet stain. **B.** Prominent hemorrhagic tear (*) in the white matter below the contralateral dural opening is present, and an increased density of glia is seen throughout the surrounding white matter. Cresyl violet stain. **C.** Reactive astrocytes labeled with GFAP immunohistochemistry are evident in the white matter surrounding the tear and the overlying gray matter.

sent in sham controls. Together, these observations suggest that contralateral axonal abnormalities are not a nonspecific consequence of the preparation procedures but are instead the result of modulating the intracranial tissue deformation to produce axonal injury. In cases where the contralateral dura mater remained intact, it is probable that the mechanical stiffness of the dura membrane prevented the necessary deformation of brain tissue in the contralateral cortex.

The lesions produced at the site of impact in this study were characterized by superficial neuronal loss, possibly associated with the small subdural hematoma produced at the time of impact. No extensive contusions or lacerations were seen in these tests, unlike the damage reported by others using the cortical impact technique (Dixon et al., 1991; Lighthall, 1988). The minimal contusion observed in the present study is likely the consequence of the relatively superficial (1.5 mm) indentation used in the animal tests. However, these differences may also be due to the differences in the geometry of the indentor tip between our device and other models, the repositioning of the impact site to a position lateral to the sagittal sinus, or other species-specific scaling effects. To address these issues, a detailed description of the nature and extent of tissue deformation and subsequent ipsilateral cortex injury as a function of impacter tip size, indentation depth, and impact velocity will be the subject of a future report. By altering the direction and time course of deformation, it should be possible to produce a variety of brain injuries, including subdural hematoma, cortical contusion, and axonal injury, each of which can be selected by choosing the appropriate impact parameters.

Characterizing the intracranial strain pattern caused by a modified cortical impact technique provides a beginning for understanding and identifying the regions that will be preferentially damaged by this method. An essential piece of information in this process is combining the architecture of the fiber tracts with the magnitude and direction of strain appearing in a specific region of the brain. In this investigation, the neuronal populations that are preferentially affected appear to be those that give rise to commissural and associational corticocortical projections. In comparison, absence of retraction balls in the internal capsule and the lack of degeneration of perikarya in the thalamic ventroposterior medial and ventroposterior lateral nuclei indicate that the axons of the thalamocortical projections were not subjected to sufficient levels of strain to cause permanent axonal injury. Thalamorcortical axons traverse the cortical white matter essentially perpendicular to the orientation of the maximum principal strain vector. These results suggest that axonal injury can be produced in selective populations of neurons by matching the characteristics of the local strain tensor with the trajectory of axonal populations. This interrelationship between the orientation of axonal trajectories relative to the orientation of strain fields may account for the diffuse pattern of axonal injury observed in humans, in which numerous damaged axons are present among populations of apparently spared axons.

Finally, the results of these experiments suggest that cortical impact with contralateral craniotomy does not produce severe brainstem injury, consistent with the results of the physical model experiments that indicated that herniation of surrogate brain material was substantially reduced when a contralateral craniotomy was used. Impact parameters used in this study appeared to cause very minor brainstem dysfunction, evidenced by only a very slight, transient alteration in respiratory rate, transient abnormality of the corneal reflex, and the appearance of relatively few swollen axons in the brainstem region. Moreover, no signs of subarachnoid hemorrhage in the medullary or pontine lower brainstem regions or upper cervical spinal cord were observed. The minimal evidence of brainstem damage in this model indicates an opportunity to separately investigate the pathobiologic aspects of forebrain axonal injury and the axonal abnormalities occurring from lower brainstem damage.

The results of this study demonstrate the advantages of an interdisciplinary approach in evaluating and understanding the biomechanical aspects of current and proposed animal models of brain injury. Comparisons between the deformation of surrogate brain tissue caused by each technique and the production of selective types of brain lesions offer the advantage of producing animal models that reproduce selected aspects of human closed head injury pathology. The combined biomechanical and anatomic approach should help to minimize the number of animals required to modify existing small animal TBI models for use in behavioral and therapeutic evaluations.

## ACKNOWLEDGMENTS

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Address reprint requests to:
*David F. Meaney, Ph.D.*
*Assistant Professor*
*Department of Bioengineering*
*University of Pennsylvania*
*240 S. 33rd Street*
*Philadelphia, PA 19104-6392*