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Neuroscience Letters 259 (1999) 5–8

Neuroscience Letters

# Immediate in vivo response of the cortex and the blood–brain barrier following dynamic cortical deformation in the rat

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Received 18 October 1998; accepted 19 October 1998

**Abstract**

Although it is known that the brain can be injured by mechanical forces initiated at the moment of impact during trauma, it is not clear how the physical response of the brain dictates the injury patterns that occur in experimental models of traumatic brain injury. In this study, we investigated the mechanical response of the brain to a technique that creates a focal injury in the rat brain. Using a transient vacuum pulse applied to the exposed cortical surface, we found that the displacement of the cortex and the extent of in vivo blood–brain barrier breakdown were related significantly to the vacuum pressure level. The relationship between the response of the cortex and injury pattern points towards a new opportunity for control of the distribution and extent of injury patterns in animal models through a precise understanding of the model biomechanics, as well as potential improvements in means of preventing traumatic brain injury. 

*Keywords:* Blood–brain barrier; Cerebral contusion; Brain injury; Biomechanics

The characterization of the relationships between the mechanical forces at the moment of injury and the mechanical parameters of a traumatic brain injury (TBI) model (e.g. force × distance, peak percussion pressure, impact velocity) can provide important insight into the brain motion and deformation during a mechanical insult. In turn, understanding the relationship between these deformations and the resulting tissue injury can present an opportunity to directly control the distribution of injury in experimental models through a change in the injury parameters. However, the precise modulation of injury parameters in an experimental model is not routinely studied, in part because of the difficulty in establishing the in vivo mechanical response of the brain in these models.

There are many injury markers that can be used to evaluate the influence of mechanical injury parameters on the extent and distribution of tissue injury. In this communication, we focus on the extent of in vivo blood–brain barrier (BBB) breakdown, a frequent traumatic brain injury (TBI) that is commonly produced in experimental models used to study TBI [1]. Although several studies point to the importance of the BBB in the sequelae of traumatic brain injury [2–5], it is difficult to study the biomechanics of BBB opening in animal models because these models have complex patterns of pathology [6]. In this report, we use a technique to focally deform the cortex, avoiding the deformation of remote regions that can occur in other experimental techniques to produce brain injury [7,8]. Moreover, we focus on BBB disruption immediately following injury, allowing us to examine how the extent of opening is related to the mechanical response of the brain.

We studied the immediate opening of the BBB by exposing the cortex to a dynamic vacuum pulse of clinically relevant (<100 ms) duration [9,10]. This technique, termed dynamic cortical deformation (DCD), induces a displacement of the cortical surface with the applied pressure (Fig. 1A). Although the in vivo nature of the technique prohibited visualization of the intracranial deformation, we measured the mechanical response of the cortex to DCD, non-invasively with a calibrated laser displacement transducer (Omron, Schaumburg, IL) positioned directly above the exposed cortex.

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0304-3940/99/$ - see front matter 
*PII* S0304-3940(98)00853-2

To study the in vivo relationship among applied pressure, cortical displacement and breakdown of the BBB, adult male Sprague–Dawley rats (350–400 g) were prepared for DCD in a manner similar to lateral fluid percussion [11]. All procedures for these experiments were approved by the University of Pennsylvania's Institution for Animal Care and Use Committee. After the animals were anesthetized (sodium pentobarbital, 60 mg/kg, i.p.), temperature was maintained at 38°C, and a 5 mm craniectomy was performed over the left parietal cortex. The dura was removed in the region of the craniectomy and an air tight Leur-Lock fitting was attached over the craniectomy with a cyanoacrylate adhesive and dental cement.

The animals were given an i.v. dose of 2% Evans Blue dye in saline (2 ml/kg) which binds quickly to serum albumin [12]. To investigate the effects of the loading conditions applied during DCD on BBB breakdown, a $3 \times 3$ experimental test matrix was designed. Individual groups of animals ($n = 7$) were injured with a 'half-sinusoidal' vacuum pulse of either 2, 3, or 4 p.s.i. over a time period of either 25, 50, or 100 ms (approximately 12.5, 25 and 50 ms rise times, respectively). An additional group of animals ($n = 7$) served as sham controls for the surgical procedures.

In general, the displacement of the cortex measured by a laser transducer lagged slightly behind the applied vacuum pressure during an experiment (Fig. 1A). The cortex often did not return to its pre-injury position, but rather demonstrated a degree of long-term or permanent displacement, most likely from tissue tears induced by the vacuum pressure. For some experiments, the laser transducer failed to produce a legible trace; this was accounted for by adjusting the sample sizes for the statistical analysis of the displacement data. The magnitude of applied vacuum pressure significantly affected cortical displacement ($P = 0.001$, Fig. 1B) (two-way ANOVA). For the 50 and 100 ms durations, linear regression analysis showed that the displacement was significantly ($P < 0.02$) related to vacuum pressure (0.33 mm/p.s.i. ($R = 0.49$) and 0.46 mm/p.s.i. ($R = 0.67$) for 50 and 100 ms durations, respectively). Although the displacement also tended to increase with duration of applied pressure, this trend was not significant.

To study the extent of BBB opening, animals were euthanized at 10 min post-injury with a lethal dose of sodium pentobarbital. This represented the earliest feasible time point for sacrifice. Previous studies at similar survival time points found minimal extravasation across the BBB [2,5,13], although the BBB became more susceptible to secondary insults at later survival times. Therefore, we believe that, while some of the extravasation at 10 min may be due to secondary insults, the majority of damage at this time point could be attributed primarily to the mechanical input.

Following sacrifice, the brains were removed and cryoprotected, and a 2 mm brain block was cut around the injury site. Previous studies of the neuropathology associated with this model, as well as preliminary studies of BBB damage ($n = 20$), indicated that all injury would be restricted to this volume of tissue. Coronal sections (300-$\mu$m thick) were cut from the block on a freezing microtome such that consecutive slices were 500 $\mu$m apart. Slices were then mounted on slides and coverslipped. We utilized the autofluorescent property of Evans Blue [14] to visualize the extent of BBB breakdown in the injured brains. Composite images of each coronal section were built using an automated imaging system (Metamorph, University Imaging, West Chester, PA) attached to an to an epifluorescence microscope (dual FITC/Texas Red barrier filter block: 480 nm excitation, 575 nm emission). To quantify the extent of BBB damage, the volume of Evans Blue-albumin extravasation was calculated for each brain. Based on the threshold pixel value for tissue taken from the rostral extent of the brain, where no neuropathological injury or BBB damage (preliminary studies, $n = 20$) was observed, we measured the area of extravasation in each slice. Volume of extravasation in a brain was then calculated by multiplying the extravasation area from an individual slice by the linear distance between slices (500 $\mu$m) and summing across all slices from a brain.

Examination of injured brains revealed Evans Blue-albumin extravasation only at the injury site (Fig. 2A). For all injury levels, extravasation volumes were significantly

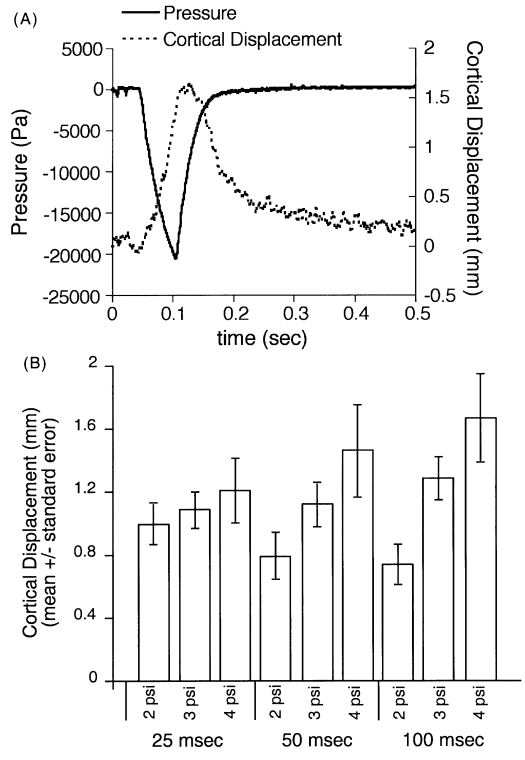


Fig. 1. (A) Pressure applied to the cortical surface (solid line) and the resulting cortical displacement (dashed line) from one of the experiments (3 p.s.i., 100 ms). The displacement lagged slightly behind the vacuum pressure, and displayed residual deformation. (B) Mean peak displacement (±SE) of the cortical surface inferior to craniectomy for each of the nine loading conditions. A two-way ANOVA revealed that vacuum pressure magnitude had a significant effect on displacement ($P = 0.001$).

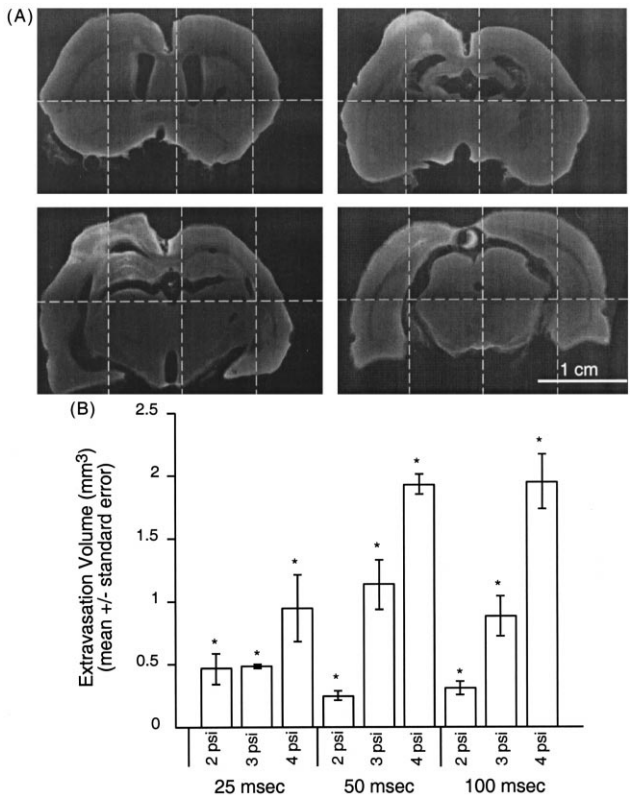


Fig. 2. (A) Composite images of coronal slices of the same brain under epifluorescence microscopy taken from the experimental DCD study (4 p.s.i., 100 ms). Bright regions depict Evans blue-albumin extravasation. Extravasation was observed directly inferior to the craniectomy in the ipsilateral hemisphere. (B) Results of the quantitative analysis of the extent of BBB breakdown following DCD. All loading conditions produced extravasation volumes produced extravasation volumes greater than surgical shams (*$P < 0.02$). A two-way ANOVA revealed that vacuum pressure magnitude ($P = 0.0005$), pulse duration ($P = 0.001$), and their interaction ($P = 0.005$) were significant factors. Scheffe's post-hoc test demonstrated that extravasation volume increased incrementally with magnitude ($P = 0.001$). For the 50 and 100 ms durations, linear regression analysis showed that the displacement was significantly ($P < 0.02$) related to vacuum pressure (0.33 mm/psi ($R = 0.49$) and 0.46 mm/psi ($R = 0.67$) for 50 and 100 ms durations, respectively).

greater than sham controls (pairwise comparisons, $P = 0.02$; Fig. 2B). In three of seven sham cases, minimal extravasation was evident in the superficial layer of the cortex, most likely due to swelling induced by exposing the cortex to atmospheric pressure. However, for all injured brains, the area of extravasation included and extended beyond the area of damage in these sham cases. Most injured brains also demonstrated tissue tears at the gray-white matter junction directly inferior to injury site. The volume of BBB was affected significantly by both the magnitude ($P < 0.001$) and duration ($P = 0.001$) of applied pressure (two-way ANOVA). Post-hoc analysis of the data revealed that, whereas volume increased incrementally from 2 to 4 psi vacuum pressure magnitude ($P = 0.001$), significant increases in response to changes in onset rate/duration were only demonstrated from 25–50 ms ($P = 0.02$; Scheffe's post-hoc test).

The specificity of BBB breakdown with this technique is different than other experimental models of TBI, and is due principally to the biomechanical features of DCD. For most other loading conditions in experimental models of brain injury, such as fluid percussion, controlled cortical impact and weight drop, a considerable shear deformation gradient is produced at the mechanical loading site [8,15,16], leading to changes in BBB permeability in this region. However, the local compression of the cortical surface during percussion or impact also causes global tissue movement and strain, thereby causing a more multi-focal injury pattern throughout the brain. Unlike the cortical surface compression that occurs in percussion or direct impact techniques, DCD causes a transient upward displacement of the cortical surface. The pressures used to cause this upward displacement are well beneath the published thresholds for contre-coup injury [17] and therefore, the highly localized elevations of shear stress and strain caused by the cortical displacement in DCD [18] are likely responsible for the BBB damage.

It is worthwhile noting that the local response of the cortex in DCD is affected also by the presence or absence of the overlying dura mater membrane. In a model using similar pressure loading conditions (suction impact [19]) but applied instead to the dura membrane, no immediate traumatic damage to the BBB was observed, but effects on the BBB and regional cerebral blood flow were reported 30 min after injury. The less severe primary injury is likely due to the stiffer dura membrane restricting the displacement of the cortex at a given pressure level in suction impact, in turn reducing the tissue stress/strain and damage at the loading site.

Relating the parameters of DCD, the applied vacuum pressure and period over which this pressure occurs, to the mechanical response of the brain allows one to estimate the mechanical measures that cause BBB injury. Comparing the measured displacement response of the cortex to the changes in extravasation volume across the loading parameters suggests that injury to the BBB is dependent on the applied deformation of the tissue, and is less influenced by the applied tissue stress. Specifically, for the same magnitude of input stress, the brain will seem more compliant for an input at a lower rate, and therefore experience more deformation. We also saw increased injury at lower input rates. This result is consistent with other cerebrovascular elements, namely the injury strain rate invariance demonstrated by subdural vessels [20]. Like other biological soft tissues, the brain exhibits a viscoelastic response to a step increase in stress or strain. A computational model of the brain motion and distortion under these experimental loading conditions confirms that these injury patterns correspond with changes in the tissue strains surrounding the loading area [17].

In the context of other animal models, the strain-based failure of the BBB suggests that controlling the extent of

BBB breakdown in animal models can be achieved by controlling the deformation of brain tissue during percussion or direct impact. For example, changing the shape of an indentor in cortical impact to maximize the strains around the impactor periphery could increase the extent of BBB breakdown. Likewise, increasing the fluid volume load during fluid percussion would increase the local and remote tissue strains, thus increasing the extent of primary BBB breakdown. These changes in the injury pattern, once the exact tissue injury criteria are known, point towards a new opportunity of modulating the extent and distribution of injury patterns in existing animal models of traumatic brain injury.

We would like to thank Dr. Andres Garcia and Dr. David Boettiger from the University of Pennsylvania for use of their epifluorescence microscopy system. Funding was provided by NIH RO1 35712, PO1 NS-08803, and CDC R49/CCR312712.

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