INTRODUCTION
Cerebral blood vessels are critical in maintaining the health and function of the brain, but their function can be disrupted by traumatic brain injury (TBI), which commonly includes damage to these vessels [1]. However, even in cases where there is not apparent mechanical damage to the cerebral vasculature, TBI can induce physiological disruptions that can lead to breakdown of the blood brain barrier or loss of cerebral autoregulation.

The walls of cerebral blood vessels are a composite of several physical structures, each with their own physiological role in maintaining structural stability and function. As a result, injury thresholds will not necessarily be the same for every component of the vessel wall. Structural failure values have been defined in the axial direction [3]. Further study, however, is needed to better characterize physiological response of vessels to sub-failure loading, as well as thresholds of deformation leading to dysfunction in the various components of the vessel wall.

As rats are commonly used in TBI research, this study aims to investigate the effect of mechanical deformation on the function of isolated rat middle cerebral arteries (MCAs), with the hypothesis that the endothelial response is disrupted prior to the smooth muscle function. Prior investigations of rat MCA function in response to injury have focused on testing of vessels after removal from an injured animal [e.g. 4,5]. As a result, the mechanical deformation the vessels experienced is unclear in these studies. Further, exposure of the MCA to the injured milieu in the brain prior to testing confounds any possible effects of mechanical deformation.

METHODS
Sample Preparation
The MCA was dissected from 6 male Sprague Dawley rats (407 ± 81 grams). Rats were euthanized via isoflurane overdose and decapitation. The brain was immediately removed and placed in a 5°C physiological saline solution (PSS) for dissection of the MCA. MCA side branches were ligated with individual fibrils from unwound 6-0 silk suture. The MCA was then cannulated with glass needles approximately 200 μm in diameter and secured with 11-0 monofilament nylon suture.

Test Set-up
The needles on which the MCA were mounted were oriented horizontally in a temperature-controlled bath, filled with PSS and maintained at 37°C. This bath had a glass window in the bottom for light and a submersible glass viewing window above the MCA. The distal needle was stationary and the proximal needle was mounted to the tester via a horizontal, low friction sled which was connected to a voice coil actuator. Movement of this voice coil moved the proximal needle horizontally along the sled track, axially stretching the MCA.

The MCA was viewed via a high magnification digital video camera in order to record the change in vessel dimensions during testing. The MCA was perfused with warm PSS originating from an open syringe hanging at the appropriate height to provide static fluid pressure. The fluid path passed through the proximal needle, mounted MCA, and the distal needle. Inline pressure transducers were located both proximal and distal to the mounted MCA, equidistant from the vessel. The average between these two transducers was taken to be the pressure inside the MCA, or the luminal pressure. Data and video acquisition, as well as control of the set-up, were accomplished via a custom LabView program (National Instruments).

Test Procedure
Vessel Equilibration
After mounting the MCA, the luminal pressure was slowly raised from 2 kPa to the testing pressure of 6 kPa in 0.5 kPa increments every 5 minutes. Once the MCA was up to pressure, it was allowed to equilibrate for 40 minutes in order to develop basal smooth muscle tone. Vessels which failed to develop
basal tone or that leaked were discarded. The MCA was gradually stretched to approximately in vivo length ($\lambda_{iv}$) during initial pressurization [3]. Throughout the entire duration the MCA was mounted in the system, the PSS in the bath was changed at least every 20 minutes.

**Characterization Tests** In order to determine the level of function in both the endothelial and smooth muscle cells, MCAs were subjected to either serial acetylcholine (ACh) and sodium nitroprusside (SNP) dose response tests or to K$^+$ dose response tests. To test the functionality of the endothelium, ACh and SNP were separately applied to the bath of 2 MCAs in serially increasing doses (ACh: $10^{-8}$ M, $10^{-6}$ M, $10^{-4}$ M; SNP: $10^{-8}$ M, $10^{-7}$ M, $10^{-6}$ M). After 2-3 minutes of exposure at each concentration, diameter was stable, and MCA inner diameter was recorded. Following either an ACh or SNP test, the bath was rinsed with fresh PSS and then refilled and allowed to equilibrate for either 20 (ACh) or 30 (SNP) minutes to allow the basal tone to redevelop. The SNP tests also served to measure the dilatational ability of the MCA smooth muscle.

To test the contractile functionality of the smooth muscle, dose response tests to K$^+$ were administered to 2 additional MCAs. Serially increasing doses of K$^+$ were applied externally by changing the PSS in the bath for PSS with altered K$^+$ concentrations but equivalent osmolarity. After 5 minutes of exposure to each K$^+$ concentration (30 mM, 60 mM, 100 mM), the inner diameter was measured. Following a K$^+$ dose response test, the bath was rinsed with fresh PSS and then refilled and allowed to equilibrate for either 20 (ACh) or 30 (SNP) minutes to allow the basal tone to redevelop. The SNP tests also served to measure the dilatational ability of the MCA smooth muscle.

**Injury Stretch** Following the baseline functional characterization via serial ACh and SNP or K$^+$ response tests, and a subsequent re-equilibration period, MCAs were stretched rapidly (strain rate $\approx 21.5$ s$^{-1}$) to an axial stretch level of $\lambda \approx (1.2)\lambda_{iv}$. The characterization test previously performed on that MCA was then repeated. Following these tests and another re-equilibration period, the MCAs were stretched again to the same stretch level but at a lower strain rate ($\approx 0.21$ s$^{-1}$). The characterization tests were then repeated again. Following the last characterization test, the bath was filled with calcium free PSS and the MCA was allowed to dilate for 30 minutes to obtain a maximum diameter measurement. If any subsequent tone was detected, SNP ($10^{-7}$ mM) was added to the bath to ensure complete dilation. To ensure that any changes following injury were not simply due to degradation of the MCA in the test system, time matched control tests were performed without any stretches (ACh and SNP tests on 1 MCA and K$^+$ tests on another), over the same time course.

**Data Analysis**

The level of response of the MCA to ACh and SNP was quantified via percent dilation, calculated as:

$$\%Dil = \frac{(D_{M} - D_{B}) - (D_{M} - D_{C})}{(D_{M} - D_{B})} \times 100$$  \hspace{1cm} (1)

where $D_{M}$ is the maximum dilated inner diameter, $D_{B}$ is the inner diameter at beginning of that test, and $D_{C}$ is the current measured inner diameter. The results of K$^+$ dose response tests were quantified using percent contraction, calculated as:

$$\%Contraction = \frac{(D_{M} - D_{C})}{D_{M}} \times 100.$$  \hspace{1cm} (2)

where 100% would be a closed lumen and 0% would be a fully dilated lumen.

**RESULTS**

Following a rapid stretch, the response of the MCAs was relatively unchanged. As illustrated in Fig. 1, percent dilation with increasing concentrations of both ACh and SNP was relatively consistent before and after rapid stretch. Percent contraction with K$^+$ was also unchanged following rapid stretch. However, the subsequent slower stretch, to the same stretch level, resulted in a blunted endothelium-dependent dilation, as seen in the reduced effect of the ACh on the MCAs following slow stretch. The slow stretch did not seem to affect the MCA response to either SNP or K$^+$ exposure. The response of the control vessels was repeatable over the time course of testing (not shown here), indicating that any changes in vessel behavior during stretch tests were not due to tissue degradation due to time in the test system.

**DISCUSSION & CONCLUSIONS**

More data are clearly needed, but preliminary findings support the hypothesis that endothelial response is disrupted before smooth muscle function following supra-physiological axial stretch. This loss of endothelial function could lead to impairment of cerebrovascular autoregulation and exacerbate injury in a head-injured subject, without structural failure of the cerebrovasculature. Interestingly, this mechanically induced endothelial dysfunction seems to be strain rate dependent, with greater damaged caused by the slower strain rate.

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**REFERENCES**


