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Quantitative analysis of brain microstructure following mild blunt and blast trauma

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ABSTRACT

We induced mild blunt and blast injuries in rats using a custom-built device and utilized in-house diffusion tensor imaging (DTI) software to reconstruct 3-D fiber tracts in brains before and after injury (1, 4, and 7 days). DTI measures such as fiber count, fiber length, and fractional anisotropy (FA) were selected to characterize axonal integrity. In-house image analysis software also showed changes in parameters including the area fraction (AF) and nearest neighbor distance (NND), which corresponded to variations in the microstructure of Hematoxylin and Eosin (H&E) brain sections. Both blunt and blast injuries produced lower fiber counts, but neither injury case significantly changed the fiber length. Compared to controls, blunt injury produced a lower FA, which may correspond to an early onset of diffuse axonal injury (DAI). However, blast injury generated a higher FA compared to controls. This increase in FA has been linked previously to various phenomena including edema, neuroplasticity, and even recovery. Subsequent image analysis revealed that both blunt and blast injuries produced a significantly higher AF and significantly lower NND, which correlated to voids formed by the reduced fluid retention within injured axons. In conclusion, DTI can detect subtle pathophysiological changes in axonal fiber structure after mild blunt and blast trauma. Our injury model and DTI method provide a practical basis for studying mild traumatic brain injury (mTBI) in a controllable manner and for tracking injury progression. Knowledge gained from our approach could lead to enhanced mTBI diagnoses, biofidelic constitutive brain models, and specialized pharmaceutical treatments.

1. Introduction

In the United States alone, over 1.7 million individuals sustain a traumatic brain injury (TBI) each year. TBI is a predominant factor in 30.5% of all injury-related deaths (Faul et al., 2010) and generates over $60 billion in medical costs (Finkelstein et al., 2006). Although the severity of TBI varies, cases of mild TBI (mTBI) are more common (Bigler and Maxwell, 2012a) especially with military personnel frequently exposed to improvised explosive devices (IEDs) (Appelboom et al., 2012). Diagnosis of mTBI is challenging because clinical signs can be physical, behavioral, cognitive, and emotional (McCrory et al., 2009). Furthermore, the specific causes of mTBI are difficult to identify because soldiers can experience blast trauma from an IED pressure wave and blunt trauma from subsequent head impacts. After experiencing mTBI, soldiers typically return to full duty status immediately after clinical signs begin to disappear, but returning prematurely can increase the risk for repeat injury (MacGregor et al., 2011). Previous studies have shown that mTBI patients experience complications associated with post-concussive syndrome (PCS) (Schneiderman et al., 2008; Belanger et al., 2010; Bryant et al., 2010) or post-traumatic stress disorder (PTSD) (Hoge et al., 2008; Carlson et al., 2010; Levin et al., 2010; Carlson et al., 2011). The underlying mechanisms of these symptoms warrants further investigation and has significant clinical implications (Budde et al., 2011).

Accurate diagnosis of mTBI is challenging because immunohistochemical techniques are invasive (Li et al., 2011) and standard MRI or CT may be insufficient (Arfanakis et al., 2002; Huisman et al., 2004). Conversely, diffusion tensor imaging (DTI) provides enhanced visualization of tissue microstructure (Sundgren et al.,

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2004) such as spinal cord (Ducreaux et al., 2007; Ozanne et al., 2007), myocardium (Wu et al., 2007; Zhang et al., 2010), and skeletal muscle (Heemskerk et al., 2007; Lansdown et al., 2007). DTI has also been utilized in the brain to assess the integrity of white matter fiber tracts (MacDonald et al., 2007a,b). Various animal models of closed-head injuries (i.e. blunt trauma) have exhibited pathological features that were also observed after blast TBI (i.e. blast trauma) (Margulies and Hicks, 2009). Some researchers argue that blunt and blast trauma generate different temporal and spatial pathological outcomes (Ling et al., 2009). However, other investigators suggest that these two sources of injury are comparable since they lead to similar cognitive consequences (Belanger et al., 2009). Although the distinctions between blunt and blast trauma remain unclear (Elder and Cristian, 2009; Cernak and Noble-Haeusslein, 2010; Hicks et al., 2010), effective comparisons can be achieved by examining the brain microstructure through DTI and image analysis.

In this study, we induced mild blunt and blast trauma in Sprague-Dawley rats with a custom-built device. We also employed DTI to track injury progression after 1, 4, and 7 days using quantitative measures including fiber count, fiber length, and fractional anisotropy (FA). Post-injury times were based on previous studies (Barkhoudarian et al., 2011; Bigler and Maxwell, 2012b) and were chosen because DTI can detect subtle brain damage linked to secondary injury (Shenton et al., 2012). Following injury and excision, we performed image analysis by using in-house software (ImageAnalyzer v2.2-0, CAVS, Starkville, MS), which comprises MATLAB scripts that are designed to quantify the defects in images of the brain's microstructure. Despite numerous studies involving animal models of TBI, few have coupled DTI with quantitative analysis of histological images to differentiate the underlying effects of blunt and blast trauma on the brain microstructure within the milder regime of injury. Thus, our goals are to determine not only if, but also why the brain responds uniquely to these two sources of injury. This study will provide further insight into whether blunt or blast trauma plays a more significant role in the progression of mTBI. A clearer understanding of this distinction could ultimately lead to more biofidelic constitutive models of the brain, more accurate diagnoses of mTBI patients, and targeted pharmaceutical treatments.

2. Methods

2.1. Animals

A total of 28 male Sprague-Dawley rats (300–325 g, Harlan Laboratories) were used in this study. Animals were housed in cages under a 12 h dark/light cycle with access to food pellets and water ad libitum and were given 3–7 days to acclimate before testing. All procedures were approved by the MSU Institutional Animal Care and Use Committee (IACUC). Animals were assigned to one of seven groups ($n=4$) with Group 1 comprising the controls. Groups 2, 3, and 4 were exposed to blunt trauma and scanned at recovery times of 1, 4, and 7 days, respectively. Groups 5, 6, and 7 were exposed to blast trauma and scanned at recovery times of 1, 4, and 7 days, respectively.

2.2. Experimental setup

Rats were anesthetized using dexmedetomidine (0.05–0.1 mg/kg, IP) and fentanyl (0.1 mg/kg, IP) followed by propofol (100 mg/kg, IP) once loss of righting reflex was observed. Fully sedated rats (i.e. loss of toe pinch reflex) were secured onto a test stage with their heads positioned over polyurethane foam, which had a thickness of 15.875 mm (0.625 in.) and density of 80 kg/m^3 (5 lb/ft^3). A marker was placed halfway between the medial canthus of both eyes to identify the impact site.

The test device included a pressure vessel (Buckeye Fabricating Co., Springboro, OH) and a pneumatic actuator (Valtorc International, Kennesaw, GA), which released compressed nitrogen once the four-way control valve was activated. Blunt injury experiments featured an air cylinder with a 1.9 cm (3/4 in.) bore diameter and 7.6 cm (3 in.) stroke (Fig. 1A). The air cylinder included a custom impactor tip and an A401 FlexiForce sensor (Tekscan, Boston, MA), which connected to a multifunction DAQ device (NI USB-6351, National Instruments, Austin, TX) capable of acquiring 1.25×10^6 samples per second. Parameters such as impact depth and impact velocity were determined from preliminary experiments designed to identify the threshold for inducing severe injuries with the test device. CT scans were used to detect skull fracture while H&E staining was employed to identify severe pathology (e.g. hemorrhage). Groups 2–4 were exposed to an impact depth of 1 mm and impact velocity of 2.2 m/s from a cylindrical indenter with a

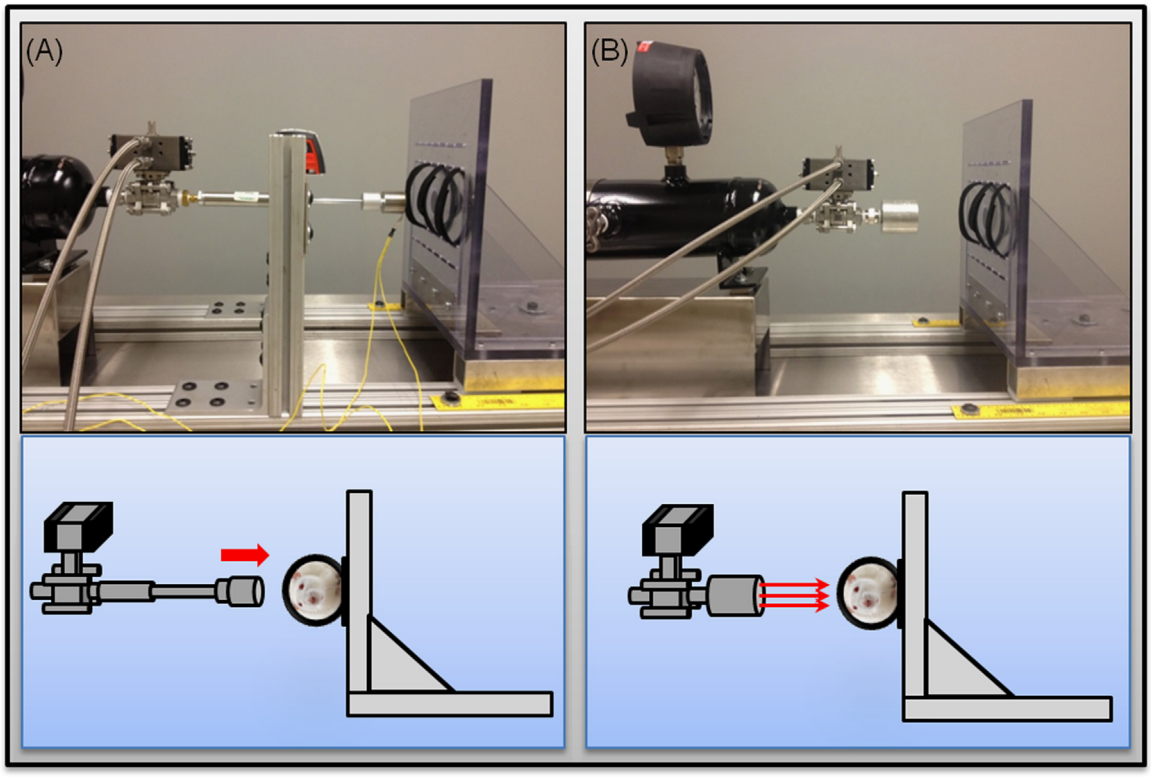

Fig. 1. Photograph and diagram of test device for (A) blunt trauma and (B) blast trauma.