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Research Paper

Adaptive reorganization of retinogeniculate axon terminals in dorsal lateral geniculate nucleus following experimental mild traumatic brain injury

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ABSTRACT

The pathologic process in traumatic brain injury marked by delayed axonal loss, known as diffuse axonal injury (DAI), leads to partial deafferentation of neurons downstream of injured axons. This process is linked to persistent visual dysfunction following mild traumatic brain injury (mTBI), however, examination of deafferentation in humans is impossible with current technology. To investigate potential reorganization in the visual system following mTBI, we utilized the central fluid percussion injury (cFPI) mouse model of mTBI. We report that in the optic nerve of adult male C57BL/6J mice, axonal projections of retinal ganglion cells (RGCs) to their downstream thalamic target, dorsal lateral geniculate nucleus (dLGN), undergo DAI followed by scattered, widespread axon terminals loss within the dLGN at 4 days post-injury. However, at 10 days post-injury, significant reorganization of RGC axon terminals was found, suggestive of an adaptive neuroplastic response. While these changes persisted at 20 days post-injury, the RGC axon terminal distribution did not recovery fully to sham-injury levels. Our studies also revealed that following DAI, the segregation of axon terminals from ipsilateral and contralateral eye projections remained consistent with normal adult mouse distribution. Lastly, our examination of the shell and core of dLGN suggested that different RGC subpopulations may vary in their susceptibility to injury or in their contribution to reorganization following injury. Collectively, these findings support the premise that subcortical axon terminal reorganization may contribute to recovery following mTBI, and that different neural phenotypes may vary in their contribution to this reorganization despite exposure to the same injury.

1. Introduction

Traumatic brain injury (TBI) is an important public health issue which continues to be a major source of death and disability for healthy adults despite strides in education, prevention, and safety. Mild TBI (mTBI) comprises up to 80% of all TBI cases and many of these patients take up to 6 months to recover from associated disability, with a minority of patients (5–10%) never fully recovering (Bazarian et al., 2005; Cassidy et al., 2004; Corrigan et al., 2010; Dean et al., 2015). Through several studies, the morbidity associated with mTBI has been associated with the presence and extent of diffuse axonal injury (DAI) found throughout the subcortical white matter and callosal projections (Christman et al., 1994; Dean et al., 2015; Farkas et al., 2006; Johnson et al., 2013; Kelley et al., 2006; King, 1997; Wolf and Koch, 2016). Of particular note in the pathogenesis of DAI, is that following axonal injury, the axon shaft distal to the injury undergoes degeneration and distal target neurons lose part of their excitatory or inhibitory input. This loss of input is hypothesized to lead to dramatic remodeling in surviving connections, however, literature supporting this premise following TBI is sparse because of the technical challenges associated with following terminal loss and recovery in a diffusely deafferentated network (Büki and Povlishock, 2006; Leunissen et al., 2014; Povlishock and Katz, 2005; Wolf and Koch, 2016).

While multiple brain loci have been linked to the morbidity associated with mTBI, visual circuit dysfunction has been recognized to be a significant contributor to morbidity in the context of mild TBI or concussion (Alvarez et al., 2012; Fimreite et al., 2015; Kapoor et al., 2004; Lachapelle et al., 2008; Lutkenhoff et al., 2013; Schlageter et al., 1993). Veterans returning from recent foreign conflicts contain a large cohort of patients with visual symptoms due to blast induced TBI (Hoge et al., 2008). Scalp recordings in post-concussive patients have also demonstrated changes in timing and amplitude of electrical potentials evoked by visual stimulus of increasing complexity as well as decreased luminosity (Fimreite et al., 2015; Papathanasopoulos et al., 2008; Yadav and Ciuffreda, 2013; Yadav et al., 2014). Collectively,

Abbreviations: AP, anterior posterior; cFPI, central fluid percussion injury; CTB, cholera toxin β; DAI, diffuse axonal injury; dLGN, dorsal lateral geniculate nucleus; mTBI, mild traumatic brain injury; VGLUT2, vesicular glutamate transporter 2.

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these reports highlight pervasive symptoms in the visual system along with cognitive and memory disturbances associated with mTBI; the latter two of which have previously received significantly more attention (Dymowski et al., 2015; Finnanger et al., 2013).

Because the detailed analysis of DAI and its correlation to morbidity and recovery are impossible to evaluate in humans using current techniques, in the current communication, we move to an animal model of mTBI. To study this question, we exploit the well-characterized visual axis of the mouse to assess deafferentation and recovery following DAI (Bickford, 2015; Bickford et al., 2010; Guido, 2008; Huberman and Feller, 2008). Using the mouse central fluid percussion injury (cFPI) model of mild TBI to induce diffuse axonal injury, we have previously reported that the optic nerve reveals a predilection for diffuse axonal injury based on identification of scattered axonal swellings as well as disconnected axonal segments (Wang et al., 2011). Distinct from other modes of optic nerve injury such as cutting, crushing, or stretching, the cFPI model induces only scattered DAI pathology with the sparing of a large fraction of axons, closely approximating the situation found in most cases of concussion and mild TBI (Marklund, 2016; Maxwell et al., 2015; Shultz et al., 2016). In the experiments described here, we utilize this model to evaluate the downstream changes evoked by injury of the optic nerve and conduct analysis of deafferentation as well as potential reorganization of retinogeniculate axon projections to the dorsal lateral geniculate nucleus (dLGN), a critical structure in the formed vision pathway of mouse.

Through the implementation of anterograde tract tracing of retinal ganglion cells (RGCs) using Alexa fluorescent dyes conjugated to recombinant cholera toxin subunit β (CTB) as well as immunohistochemistry against a well described marker for retinorecipient axon terminals, vesicular glutamate transporter 2 (VGLUT2), we examined deafferentation of dLGN relay neurons through the detection of retinogeniculate axon terminals loss and recovery. Following cFPI induced DAI in the adult mouse optic nerve, we found axon terminal loss occurred in its downstream target, the dorsal lateral geniculate nucleus (dLGN). However, over time, the remaining intact axon fiber population underwent structural reorganization of pre-synaptic terminals in a manner consistent with axon terminal sprouting and adaptive recovery. Our findings support the hypothesis that in the adult mammalian brain, surviving subcortical axons and their axon terminals are capable of dramatic reorganization following mTBI induced DAI. We believe that these findings have important implications for our understanding of DAI and its consequences in humans, while providing important insight into the repair and restoration of function following mTBI.

2. Materials and methods

2.1. Experimental design

Adult male C57BL/6J mice (9–12 weeks) were sourced directly from Jackson Laboratory (Bar Harbor, ME) or bred and maintained in house. Animals obtained directly were kept in house for 48 h to habituate to our vivarium prior to experiments. Animals were then subjected to mild central fluid percussion injury (1.40 ATM ± 0.05), as previously described (Greer et al., 2013; Hånell et al., 2015; Wang et al., 2011, 2013). In brief, the fluid pressure pulse in this model results in a brief deformation of the brain through an intact dura and induces diffuse traumatic axonal injury in the optic nerve approximately 1 mm proximal to the chiasm as well as other neocortical and subcortical sites not addressed in the current study.

As employed, the model involved the induction of general anesthesia in adult male mice with inhaled isoflurane (<5%) before placing their head in a stereotactic frame. Toe pinch reflexes were monitored during surgery and isoflurane concentration adjusted to maintain depth of anesthesia. The skin was opened over the skull and retracted to allow for a 3 mm wide craniotomy midway between lambdoid and bregma cranial sutures. Care was taken to keep the underlying dura intact and a dissecting microscope was used to ensure dural integrity as well as prevention of injury to the large sagittal vascular structures. A luer hub was fixed in place over the craniotomy with dental cement and the animal was allowed to recover from anesthesia for approximately 1 h. The animal was then anesthetized (4% isoflurane) again prior to mounting to the fluid percussion device and the impulse delivered to the intact dura of the mouse brain. Sham-injured animals received the same surgeries including, craniotomy, the mounting of the luer hub and attaching the animal to the fluid percussion device, with the absence of the injury that results from release of the pendulum. During surgery to perform craniotomy and implant the luer hub, each mouse was monitored using a pulse oximeter sensor to collect blood oxygen saturation, heart rate, and respiratory rate. Post-injury or sham procedure, return of toe pinch reflex as well as righting reflex were recorded.

The animals were prepared for histologic analysis at 4, 10, and 20 days after injury. The early time point of 4 days was selected to allow axonal disconnection to reach its apogee prior to evaluation of deafferentation based on our previous report (Wang et al., 2011). We previously examined mTBI retinas for RGC death induced by DAI and demonstrated that the injured/axotomized RGCs continue to survive up to 28 days after injury (Wang et al., 2013). The time points of 10 and 20 days were chosen based on mouse studies evaluating deafferentation and recovery in the visual axis (Benowitz and Yin, 2008; Lima et al., 2012). For the histological analyses, the animals were prepared in three difference fashions for either immunohistochemistry, CTB, or routine histopathology. Animals prepared for CTB fluorophore visualization or VGLUT2 immunohistochemistry were sacrificed via intraperitoneal overdose of sodium pentobarbital. Those prepared for CTB visualization were transcardially perfused with 25 mL of heparinized 0.9% saline (1 u/mL) followed by 200 mL of 4% paraformaldehyde dissolved and filtered into Millonig's buffer (pH 7.4). Animals undergoing VGLUT2 immunohistochemistry or standard histologic preparation in plastic sections were perfused in a similar fashion with the addition of 0.2% glutaraldehyde to the fixative perfusion solution. The brain, optic nerves, and eyes of each animal were post-fixed in their respective fixative for 24 h and stored at 4 °C in Millonig's buffer until sectioning. All protocols used in this study were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

2.1.1. Examination of mTBI induced retinogeniculate axon terminal loss via VGLUT2 immunohistochemistry

For analysis of VGLUT2 immunoreactive axon terminal loss and recovery, animals were prepared as described above for mTBI (1.4 ± 0.05 ATM) or sham injury. Sham animals were prepared from littermates of mTBI animals and were sacrificed at similar time points following surgery. Our inclusion criteria were the absence of dural injury during surgical procedures, adequate wound healing following injury or sham-injury, normal feeding and drinking, and normal grooming in days following surgery. All animals prepared for VGLUT2 immunohistochemistry met inclusion criteria and therefore none were excluded from analysis. A total of 6 sham animals together with 4–5 mTBI animals for each post-injury time point were used to generate sections for analysis (Sham $N = 6$, 4 day mTBI $N = 4$, 10 day mTBI $N = 5$, 20 day mTBI $N = 5$). Free floating coronal sections (40 μm) were obtained using a vibrating blade microtome (Leica VT1000 S). We determined a randomized start point and collected alternating sections through the entire left and right dLGN for each animal. Immunohistochemistry was performed on all collected sections by a blinded investigator. Sections were serial washed in phosphate buffered saline (PBS) before incubating for 1 h at room temperature in blocking solution containing; 0.3 M glycine (Sigma-Aldrich), 2.5% bovine serum albumin (Electron Microscopy Sciences), and 2.5% normal goat serum (S1000, Vector Labs), in phosphate buffered saline (pH 7.2). Sections were then incubated overnight at room temperature on oscillating stage with 1:2000 dilution of affinity purified rabbit anti-VGLUT2 polyclonal IgG antibody (Synaptic