Molecular and Cellular Neuroscience xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

The pathophysiology of repetitive concussive traumatic brain injury in experimental models; new developments and open questions

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ARTICLE INFO

Article history:
Received 6 January 2015
Revised 3 February 2015
Accepted 6 February 2015
Available online xxxx

Keywords:
Concussion
Traumatic brain injury
Traumatic axonal injury
Amyloid-beta
Tau
Microglia
Depression
Social behavior
Mouse
Sertraline

ABSTRACT

In recent years, there has been an increasing interest in the pathophysiology of repetitive concussive traumatic brain injury (rcTBI) in large part due to the association with dramatic cases of progressive neurological deterioration in professional athletes, military personnel, and others. However, our understanding of the pathophysiology of rcTBI is less advanced than for more severe brain injuries. Most prominently, the mechanisms underlying traumatic axonal injury, microglial activation, amyloid-beta accumulation, and progressive tau pathology are not yet known. In addition, the role of injury to dendritic spine cytoskeletal structures, vascular reactivity impairments, and microthrombi are intriguing and subjects of ongoing inquiry. Methods for quantitative analysis of axonal injury, dendritic injury, and synaptic loss need to be refined for the field to move forward in a rigorous fashion. We and others are attempting to develop translational approaches to assess these specific pathophysiological events in both animals and humans to facilitate clinically relevant pharmacodynamic assessments of candidate therapeutics. In this article, we review and discuss several of the recent experimental results from our lab and others. We include new initial data describing the difficulty in modeling progressive tau pathology in experimental rcTBI, and results demonstrating that sertraline can alleviate social interaction deficits and depressive-like behaviors following experimental rcTBI plus foot shock stress. Furthermore, we propose a discrete set of open, experimentally tractable questions that may serve as a framework for future investigations. In addition, we also raise several important questions that are less experimentally tractable at this time, in hopes that they may stimulate future methodological developments to address them.
This article is part of a Special Issue entitled "Traumatic Brain Injury".

1. Traumatic axonal injury

Based on human and experimental animal data, it has been hypothesized that axons are fundamentally the most vulnerable structures in the brain to relatively mild, concussive traumatic brain injury. Specifically, in a series of autopsy cases in which the victims had recent concussive TBI and then died of other causes, Blumbergs et al. reported that traumatic axonal injury was found in 6/6 cases. Other pathological signs of TBI such as hemorrhage, contusion, and skull fracture were absent in the concussion cases, though they were readily detected in more severe TBI cases (Blumbergs et al., 1994, 1995). In a model of mouse repetitive concussive TBI, we reported electron microscopic evidence of axonal injury as well (Shitaka et al., 2011). However, most of the injured axons were not dilated, making them difficult to visualize using conventional immunohistochemistry. Thus, we reasoned that conventional light microscopic methods may substantially underestimate the extent of traumatic axonal injury. This line of reasoning was further supported by the findings that diffusion tensor imaging, an MRI method sensitive to white matter microstructure, revealed abnormal signals even in mice with controlled cortical impact injuries too mild to demonstrate substantial light microscopic immunohistochemical abnormalities (Fig. 1, adapted from Brody et al., "Current and Future Diagnostic Tools for Traumatic Brain Injury: CT, Conventional MRI, and Diffusion Tensor Imaging" Handbook of Neurology, 2015 in press). Silver staining, an indirect approach to assessing degenerating white matter, correlated only modestly with diffusion tensor abnormalities following experimental rcTBI (Bennett et al., 2012).

Thus, developing methods to efficiently assess non-dilated, injured axons following rcTBI became a top priority. We considered several approaches: (Table 1) and settled on array tomography-based immunofluorescent assessments as the most immediately promising. Array tomography involves cutting ribbons of serial ultrathin sections using an ultramicrotome, then performing immunofluorescent labeling on the ribbons (Micheva and Smith, 2007). The ultrathin sections provide

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http://dx.doi.org/10.1016/j.mcn.2015.02.005
1044-7431/

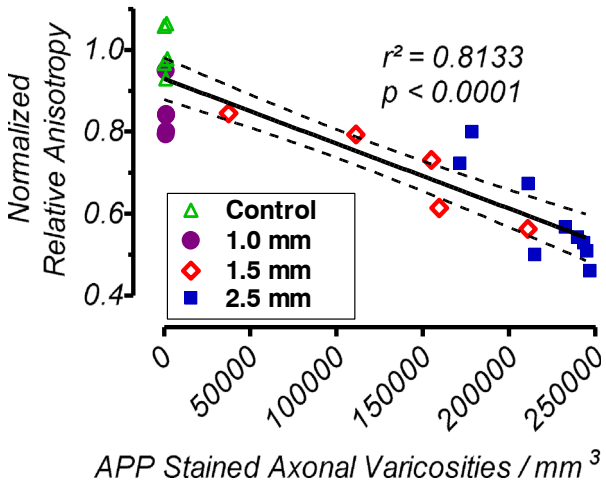

Fig. 1. Diffusion tensor imaging in a mouse model of pericontusional traumatic axonal injury: Mice were injured with controlled cortical impact at 3 different severities (1.0 mm, 1.5 or 2.5 mm impact depth), scanned with DTI 24 h later, and then sacrificed for quantitative histological assessment of axonal injury using stereological counting of APP stained axonal varicosities. Methods were otherwise as previously described (Mac Donald et al., 2007a, 2007b). Notably, relative anisotropy was reduced in the least severely injured mice (1.0 mm) even though essentially no dilated APP-immunoreactive axons were observed. Subsequent work has demonstrated the presence of non-dilated, APP-negative injured axons following less severe injuries.
Adapted from Brody et al., "Current and Future Diagnostic Tools for Traumatic Brain Injury: CT, Conventional MRI, and Diffusion Tensor Imaging" Handbook of Neurology, 2015 in press.

exceptionally good signal to noise and spatial resolution, substantially better than optical (e.g. confocal) sections. The method has been used to quantify synaptic loss in both mouse models and human tissue (Koffie et al., 2009, 2012). We adapted this technique to the study of injured white matter following repetitive concussive TBI by using the monoclonal antibody SMI32, which works well in resin-embedded sections and stains only injured axons in the mouse brain. Using largely automated image processing techniques, we determined that the mouse corpus callosum contains approximately 2000 SMI32-immunoreactive axons per cubic mm 7 days after repetitive concussive TBI (vs. essentially zero in control mice and in conventional thick sections stained with APP). Co-labeling with tubulin antibody in the same sections allowed us to determine that there were approximately 20,000 total axons per cubic mm in the region, and that the total number was not diminished by rcTBI at this time point (Bennett and Brody Journal of Neuroscience Methods 2015 in press).

2. Tau pathology

Tau pathology is a prominent finding in post-mortem assessments of boxers, American football players, military personnel and others who have suffered repetitive concussive traumatic brain injuries (Goldstein et al., 2012; McKee et al., 2009, 2012). However, the mechanisms underlying this tau pathology are not known. Our previous findings in a mouse model of more severe contusional TBI indicated that tau pathology induced by TBI may be mechanistically distinct from tau pathology due to age-related neurodegenerative diseases (Tran et al., 2011a, 2011b, 2012). Notably, much of the tau accumulation occurs in dilated axons via an amyloid-beta independent but c-jun N-terminal kinase dependent mechanism.

To date, we have not detected any effect of rcTBI on immunohistochemically apparent tau pathology in either hTau or P301S tau transgenic mice 1 week, 1 month and 6 months using conventional light microscopic analyses. For example, we tested the effects of 4 closed skull concussive traumatic brain injuries spaced 24 h apart alternating between right and left sides of the skull in 6–8 week old hTau mice (Andorfer et al., 2003). Littermates were randomly assigned to either 4 injuries or 4 sham procedures, then sacrificed 7 days later. The injuries produced substantial abnormal silver staining, indicative of injury (Fig. 2A–B). However, there was no change in the extent of phospho-tau immunoreactivity using the CP13 antibody (Fig. 2C–D), which recognizes tau phosphorylated at serine 202 and has been commonly used to assess human Chronic Traumatic Encephalopathy (CTE) pathology (McKee et al., 2012). Tau knockout mice that were littermates of the hTau animals were also assessed as negative controls. There was no difference in the extent of silver staining between injured hTau mice and injured tau knockout mice (Fig. 2B).

We have used 2 to 4 injuries 24 h apart; these experimental parameters are arbitrary, and there is no consensus regarding the optimal rcTBI model. Several other groups have also developed rcTBI models (Table 2) with widely varying experimental parameters. Most models have resulted in acute to subacute behavioral deficits and relatively subtle histological abnormalities. While there has been some tau immunostaining reported (Ojo et al., 2013; Petraglia et al., 2014b), to date none of the models have recapitulated the progressive cortical and perivascular tau pathology that defines human CTE.

Table 1
Approaches to assessing non-dilated injured axons following rcTBI.

	Advantages	Disadvantages
Quantitative electron microscopy	Highest standard of sensitivity May provide insight into mechanisms (myelin damage, cytoskeletal disruption…)	Slow, labor intensive, expensive, prone to fixation artifacts, challenging to combine with molecular specific assessments Small regions assessed Manual counting of axons required
Silver staining of conventional tissue sections	Relatively fast and inexpensive Large regions of tissue assessed	Unknown mechanism of staining Nonspecific Not fully quantitative
Superresolution fluorescence microscopy	Potentially high sensitivity Potential to provide insight into mechanisms through molecular targeted probes.	Slow Very expensive Small regions assessed Unknown artifacts Will require extensive validation
Array tomography-based immunofluorescence microscopy	More sensitive than conventional fluorescent microscopy Faster than electron microscopy and superresolution microscopy Molecularly targeted probes Larger regions assessed than EM or superresolution microscopy High signal to noise allowing automated counting of injured axons	Unknown sensitivity to the smallest injured axons More labor intensive than conventional light microscopy Approximately 50% of antibodies work well in resin-embedded ultrathin sections.

Please cite this article as: Brody, D.L., et al., The pathophysiology of repetitive concussive traumatic brain injury in experimental models; new developments and open questions, Mol. Cell. Neurosci. (2015), http://dx.doi.org/10.1016/j.mcn.2015.02.005