



A Hit, a Hit—A Very Palpable Hit: Mild TBI and the Development of Epilepsy

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Repetitive Diffuse Mild Traumatic Brain Injury Causes An Atypical Astrocyte Response and Spontaneous Recurrent Seizures

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Focal traumatic brain injury (TBI) induces astrogliosis, a process essential to protecting uninjured brain areas from secondary damage. However, astrogliosis can cause loss of astrocyte homeostatic functions and possibly contributes to comorbidities such as posttraumatic epilepsy (PTE). Scar-forming astrocytes seal focal injuries off from healthy brain tissue. It is these glial scars that are associated with epilepsy originating in the cerebral cortex and hippocampus. However, the vast majority of human TBIs also present with diffuse brain injury caused by acceleration–deceleration forces leading to tissue shearing. The resulting diffuse tissue damage may be intrinsically different from focal lesions that would trigger glial scar formation. Here, we used mice of both sexes in a model of repetitive mild/concussive closed-head TBI, which only induced diffuse injury, to test the hypothesis that astrocytes respond uniquely to diffuse TBI and that diffuse TBI is sufficient to cause PTE. Astrocytes did not form scars and classic astrogliosis characterized by upregulation of glial fibrillary acidic protein was limited. Surprisingly, an unrelated population of atypical reactive astrocytes was characterized by the lack of glial fibrillary acidic protein expression, rapid and sustained downregulation of homeostatic proteins, and impaired astrocyte coupling. After a latency period, a subset of mice developed spontaneous recurrent seizures reminiscent of PTE in human patients with TBI. Seizing mice had larger areas of atypical astrocytes compared with nonseizing mice, suggesting that these atypical astrocytes might contribute to epileptogenesis after diffuse TBI. Traumatic brain injury is a leading cause of acquired epilepsies. Reactive astrocytes have long been associated with seizures and epilepsy in patients, particularly after focal/lesional brain injury. However, most TBIs also include nonfocal, diffuse injuries. Here, we showed that repetitive diffuse TBI is sufficient for the development of spontaneous recurrent seizures in a subset of mice. We identified an atypical response of astrocytes induced by diffuse TBI characterized by the rapid loss of homeostatic proteins and lack of astrocyte coupling while reactive astrocyte markers or glial scar formation was absent. Areas with atypical astrocytes were larger in animals that later developed seizures suggesting that this response may be one root cause of epileptogenesis after diffuse TBI.

Commentary

In the United States, almost 3 million people sustain a traumatic brain injury (TBI) every year. The vast majority of these injuries are categorized as mild ($\approx 90\%$) and do not require hospitalization.¹ Mild TBI is also frequently categorized as concussion, and it remains controversial as to whether, and to what extent mild TBI is a risk for the development of post-traumatic epilepsy. It is well established, on the other hand, that severe TBI can lead to the development of a range of negative sequela in humans, including epilepsy.² Moreover, the epileptogenic effects of severe TBI have been confirmed in rodents following controlled cortical impact and fluid percussion injury.² In both rodent models, a craniectomy is performed to expose the dura, and injury is induced either by a rigid impact

device or hydraulically induced pressure. Both models can produce severe injuries, including neuronal loss, hemorrhage, extensive inflammatory changes, and mortality. These models have provided a wealth of data about potential epileptogenic mechanisms of severe TBI, but do not provide insight into the effects of mild TBI on epileptogenesis.

To address this gap in knowledge, Shandra and colleagues developed a closed-head model of post-traumatic epilepsy in mice following repetitive, mild TBI. Traumatic brain injury was induced by dropping a 100 g weight from a 50 cm height onto the anesthetized mouse's head, which rested on a foam pad. Three impacts were given at 45-minute intervals. Unimpacted controls were also anesthetized. Animals receiving impacts took about 2 minutes longer to recover from anesthesia



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than controls—suggestive of brief unconsciousness. Overall mortality was only 4% and significant neuronal loss was absent. Nine animals underwent 24/7 long-term electroencephalogram (EEG) monitoring, and 44% (4 of 9) developed either focal nonconvulsive seizures or convulsive seizures. The earliest seizure occurred 21 days after mild TBI, while the mean latency was 38 days. This is well outside the clinically defined 1 week period during which acute, nonepileptic injury-induced seizures occur following TBI. Convulsive seizures were associated with behavioral changes, including Racine scale class V “rearing and falling.”³ The combination of EEG and behavioral manifestations in these animals provides convincing evidence that the mice became epileptic.

Histological studies in the mice revealed a novel combination of astrocytic changes. Western blot studies demonstrated a modest increase in glial fibrillary acid protein, but no evidence of astrocyte proliferation. This is notable, as astrocyte proliferation typically follows more severe focal brain injuries.⁴ Animals with mild TBI, however, exhibited patches of cortex with reduced immunostaining for several astrocytic markers, including glutamate transporter 1 (Glt1), glutamine synthetase, the inward rectifier potassium channel Kir4.1, and the gap junction channel protein connexin43 (Cx43). Cell counts and cell death measures support the conclusion that astrocytes are still present in these patches but have developed phenotypic changes in protein expression. In the mild TBI animals, these atypical astrocytes covered 6% of cortex.

The astrocytic proteins downregulated in these cortical patches have been implicated in epilepsy.⁵ Glutamate transporter 1 and glutamine synthetase are responsible for the uptake and metabolism, respectively, of glutamate released from synapses. Genetic deletion of Glt1 leads to glutamate accumulation at synapses and seizures in other models,⁶ while glutamine synthetase is a key enzyme in the glutamine-glutamate-gamma aminobutyric acid (GABA) cycle. Kir4.1 channels, on the other hand, are critical for spatial buffering of potassium. Downregulation of these channels can elevate extracellular potassium levels, increasing neuronal excitability.⁷ The effects of Kir4.1 loss may be compounded by Cx43 downregulation. Channel protein connexin43 mediates gap junction coupling among astrocytes, facilitating movement of potassium through the astrocyte network from regions with high concentrations (eg, synapses) to regions with lower concentrations (eg, capillary networks).⁷ Notably, Shandra and colleagues introduced the gap junction-permeable tracer biocytin into astrocytes in tissue from the mild TBI animals, but found that the molecule was excluded from cortical patches with abnormal astrocytes. Although not directly tested here, the findings suggest that potassium will accumulate in these regions because of transporter downregulation and decoupling of astrocytes with surrounding astrocytic networks.

The appearance of potentially proepileptogenic changes among patches of cortical astrocytes is consistent with the hypothesis that these changes may play a causal role in epileptogenesis. Also consistent with this interpretation, changes appeared within 1 week; before the onset of spontaneous

seizures. Furthermore, the size of the cortical area in which Glt1 immunoreactivity was reduced positively correlated with seizure occurrence. Nonetheless, findings in the model are correlative. Future interventional studies are needed to determine whether the astrocytic changes are cause, effect, or epiphenomena. It is also important to further characterize this model for other putative pro-epileptogenic changes, such as circuit reorganization and changes in the expression of neuronal proteins. Assessment of neuronal function in the astrocytic patches exhibiting protein downregulation relative to cortical regions containing apparently healthy astrocytes will be of particular interest. The abnormal patches are predicted to exhibit potassium accumulation, glutamate accumulation, and localized hyperexcitability.

The occurrence of epilepsy in an animal model of mild, repetitive TBI heightens concerns that similar injuries could lead to epilepsy in humans. Repetitive mild TBI can occur during a variety of sports in which head impact is common. Whether mild TBI in humans increases the risk of epilepsy, however, remains controversial. While some studies have found evidence of increased risk,⁸ other have not.⁹ Since it is clear that moderate and severe TBI increases epilepsy risk, the real question here is exactly what injury threshold is required to initiate epileptogenesis? A challenge for clinical studies aimed at assessing risk certainly lies in the identification of patients and the assessment of injury severity. Many patients with presumably milder TBI likely do not seek medical attention, while standard tools for identifying mild TBI (absence of magnetic resonance imaging/computed tomography findings, Glasgow Coma Scale) have a broad range of heterogeneity. Beyond these technical issues, the biology is complex, with epileptogenic thresholds likely varying in individual patients depending on a variety of other factors. Notably, susceptibility to acute seizures in animal models is modulated by a wide range of variables, including stress, neurogenesis rates, brain developmental states, hormonal factors, and gene variants. Susceptibility to epileptogenesis in humans may be similarly modulated. Prior history of injury is also likely to be important. Specifically, Shandra and colleagues found that a 45-minute delay between impacts significantly increased recovery time, while recovery time was similar to a single impact when impacts were separated by 24 hours. This highlights the importance of inter-injury interval described by other investigators,¹⁰ but adds a further level of complexity. While clear answers will take considerably more research, the development of a mild TBI model that produces post-traumatic epilepsy provides a system in which these questions can begin to be addressed.

By Steve C. Danzer

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