



# TLR4 Signaling Contributes to Post-Traumatic Epileptogenesis Through Insertion of Inwardly Rectifying AMPA Receptors: Not a Happy CP-AMPA

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## Toll-like Receptor 4 Signaling in Neurons Enhances Calcium-Permeable $\alpha$ -Amino-3-Hydroxy-5-Methyl-4-Isloxazolepropionic Acid Receptor Currents and Drives Post-Traumatic Epileptogenesis

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**Objective:** Traumatic brain injury is a major risk factor for acquired epilepsies, and understanding the mechanisms underlying the early pathophysiology could yield viable therapeutic targets. Growing evidence indicates a role for inflammatory signaling in modifying neuronal excitability and promoting epileptogenesis. Here, we examined the effect of innate immune receptor Toll-like receptor 4 (TLR4) on excitability of the hippocampal dentate gyrus and epileptogenesis after brain injury. **Methods:** Slice and in vivo electrophysiology and western blots were conducted in rats subject to fluid percussion brain injury or sham injury. **Results:** The studies identify that TLR4 signaling in neurons augments dentate granule cell calcium-permeable  $\alpha$ -amino-3-hydroxy-5-methyl-4-isloxazolepropionic acid (AMPA) receptor (CP-AMPA) currents after brain injury. Blocking TLR4 signaling in vivo shortly after brain injury reduced dentate network excitability and seizure susceptibility. When blocking of TLR4 signaling after injury was delayed; however, this treatment failed to reduce postinjury seizure susceptibility. Furthermore, TLR4 signal blocking was less efficacious in limiting seizure susceptibility when AMPAR currents, downstream targets of TLR4 signaling, were transiently enhanced. Paradoxically, blocking TLR4 signaling augmented both network excitability and seizure susceptibility in uninjured controls. Despite the differential effect on seizure susceptibility, TLR4 antagonism suppressed cellular inflammatory responses after injury without impacting sham controls. **Interpretation:** These findings demonstrate that independently of glia, the immune receptor TLR4 directly regulates post-traumatic neuronal excitability. Moreover, the TLR4-dependent early increase in dentate excitability is causally associated with epileptogenesis. Identification and selective targeting of the mechanisms underlying the aberrant TLR4-mediated increase in CP-AMPA signaling after injury may prevent epileptogenesis after brain trauma.

## Commentary

Traumatic brain injury (TBI) is the underlying cause of more than a fifth of all symptomatic epilepsy.<sup>1</sup> Since post-traumatic epilepsy (PTE) inherently has a well-defined “start point” (ie, the time of TBI), it represents not only a well-formed experimental model for studying basic mechanisms of epileptogenesis but also a well-defined clinical point of intervention. Depending on the exact nature of the primary injury, TBI can produce an array of acute damage including subdural hematoma, intracranial hemorrhage, and axonal shearing.<sup>2,3</sup> Perhaps the most obvious and ubiquitous secondary product of TBI is the inflammatory response. This response begins with damage-associated molecular patterns resulting from the primary injury,<sup>4</sup> which recruit microglia. Damage-associated molecular pattern receptor signaling triggers the production of cytokines and chemokines which, in turn recruit peripheral immune cells.

This inflammatory response is a double-edged sword, presumably critical to recovery following brain injury, repairing injured brain tissue, and clearing toxic molecules left behind by ruptured cells.<sup>5</sup> However, it also results in acute brain swelling and promotes apoptotic pathways, which can produce substantial secondary brain injury. Current clinical relief from this inflammation is primarily accomplished through invasive decompressive surgical intervention. Clinical trials to acutely pharmacologically suppress the immune response to TBI have been ineffective or even detrimental,<sup>6</sup> perhaps because the aforementioned benefits of the inflammatory response outweigh the costs.

The failure of global immunosuppressive drugs to ameliorate the secondary effects of TBI-induced inflammation highlights the need to identify more specific drug targets for prophylactic treatments following TBI. The situation is further



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complicated by the fact that many inflammatory signals have multiple pathways, only some of which are related to PTE.<sup>7</sup> Furthermore, the ubiquity of inflammation in TBI makes it difficult to determine whether signals are causal of or simply correlated with PTE.

In the highlighted study,<sup>8</sup> Korgaonkar et al help to fill this knowledge gap by characterizing the complex role of Toll-like receptor 4 (TLR4) in PTE and its potential as a target for anti-epileptogenic treatment. Toll-like receptor 4 is a receptor found on both neurons and glia, but previously believed to modulate neuronal excitability by inducing glial release of N-methyl-D-aspartate (NMDA) receptor-enhancing cytokines. However, in the current study, the authors demonstrate that fluid percussion injury (FPI) produces an increase in neuronal excitability, in slices prepared at 1 week postinjury, that is driven by a TLR4-mediated increase in surface expression of calcium-permeable  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (CP-AMPARs), which are the inwardly rectifying (more excitable) variety of AMPAR. This pathway was definitively neuronal as TLR4 antagonists also decreased the activity of CP-AMPARs in purely neuronal cultures, which exhibit the injured AMPAR phenotype. In vivo, either systemic or focal treatment with TLR4 antagonists, beginning 1 day after FPI and continuing for 3 days, decreased the severity of PTE (as measured by latency to seizure following low-dose kainate injection). Treatment at 1 month postinjury had no effect on injured rats. Perhaps underlying the ineffectiveness of late treatment, there was no evidence of elevated surface CP-AMPAR expression in FPI versus sham animals at 1 month. Interestingly, there was elevated total GluA1 content (the surrogate marker for CP-AMPAR) in injured animals; it was just not preferentially located in the membranes. Early treatment with TLR4 antagonists significantly reduced total GluA1 levels.

Together these results suggest a model wherein TBI increases neuronal excitability and contributes to PTE via a TLR4-mediated increase in surface CP-AMPARs. If surface CP-AMPAR expression returns to normal by 1 month postinjury, one might expect seizures/excitability to wane at this time point as well, but it does not. How does transient elevation of CP-AMPARs lead to a long-term increase in neuronal excitability? And how do TLR4 antagonists, which block the transient elevation of CP-AMPARs, produce a long-term decrease in excitability? One possibility is that TBI-induced axonal injury and cell death produce widespread deafferentation, which leads to compensatory potentiation of surviving synapses. A recent study suggests that long-term potentiation involves activation of silent synapses through insertion of CP-AMPARs, which later mature into calcium-impermeable AMPARs.<sup>9</sup> This study demonstrated that calcium flux through CP-AMPARs during the early phase of potentiation was necessary to establish chronically potentiated, calcium-impermeable AMPA synapses. This is congruent with the findings in the highlighted paper that (1) TBI transiently increases membrane CP-AMPARs, but chronically enhances excitability and (2) temporarily blocking insertion of CP-AMPARs with

TLR4 antagonists prevents the establishment of a chronically excitable network (presumably by preventing consolidation of chronically potentiated synapses).

Together the effects of TLR4 antagonists on injured rats suggest a promising therapeutic regimen in which a brief course of treatment following TBI substantially reduces epileptogenesis. The biggest hurdle to translation for the TLR4 antagonists tested in the highlighted paper is that, for most assays presented, the drugs exhibited opposite effects in FPI and sham-injured animals. In sham-injured animals, TLR4 antagonists acutely *increased* excitability in vitro and chronically *lowered* seizure threshold and *increased* mortality in vivo. This is somewhat counterintuitive as one might expect a therapy that specifically reduced AMPA transmission to decrease excitability. One possibility is that TLR4 antagonists have off-target excitatory effects, unrelated to AMPARs. Another explanation could be that, in sham animals, TLR4 antagonists reduce AMPARs onto interneurons, decreasing inhibition. Alternatively, TLR4 antagonists may actually reduce CP-AMPARs in a subset of cells as expected and then produce a compensatory homeostatic response that increases excitability in the long term, though this would not explain the acute effects of TLR4 antagonists in sham controls. The mechanism of this seemingly paradoxical effect remains unclear, but uncovering it will be a critical step in developing translatable therapies for the prevention of post-traumatic epileptogenesis based on this thorough and elegant starting point from Korgaonkar et al.

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