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Axons Get Excited to Death

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Not long ago, excitotoxicity (ie, neural damage due to excessive stimulation of glutamate receptors) was a concept reserved for gray matter (GM) areas of the brain. White matter (WM), after all, was devoid of synaptic machinery and the receptors that come with it, or so we thought. The mental block against the idea of glutamate involvement in WM injury dissolved with the detection of glutamate receptors on myelinating oligodendrocytes and the startling discovery that blocking glutamate receptors protected against ischemic and traumatic injury in WM.^{1–4} But where do the offending glutamate receptors reside? And where does the damaging glutamate come from and how does it enter the extracellular space to cause its toxic mischief in WM? These questions and related ones are foremost in much of the ongoing work in this active field. Stys and colleagues in this issue of the *Annals of Neurology*^{5,6} provide provocative new findings directly relevant to the first of these questions: Axons themselves appear to express functional glutamate receptors! Their work raises at least as many questions as it answers. No matter. The hope is that all this new information about WM injury will catalyze development of effective treatments for patients at risk for WM damage due to stroke, trauma or more chronic conditions such as multiple sclerosis (MS). This can occur with or without all of the mechanistic details.

Why are we so concerned about WM injury, especially ischemic injury, in the first place? After all, highly validated stroke research on rodents indicated that selective protection of GM, primarily by blocking NMDA-type glutamate receptors, offered impressive reduction of infarct volume. It required many failed human stroke trials using drugs found promising in mice and rats before we realized that something must be different about the human brain. One critical difference, of course, is that our brains have a lot more WM than rodents, roughly five-fold more.⁷ Consequently, clinical deficits in human stroke result from damage to both GM and WM. Unless the mechanisms of irreversible tissue injury are identical for WM and GM, and they are not,⁸ we are obligated to protect, or minimize damage to, both of these unique types of brain tissue. Failure to do so will mean more disappointing clinical trials. A deep understanding of WM injury mechanisms has implications for other neurological conditions that affect WM, whether acutely as in spinal cord trauma, or more chronically as in demyelinating diseases like MS.

A thorny issue has been how to “isolate” WM for the purpose of analyzing the acute injury cascade peculiar to this special part of the central nervous system. In injury models where both axons and their neuron cell bodies of origin are insulted, failure of axon function can be due to cell body damage or diffusion of toxic molecules from GM into WM. These confounding variables can be avoided using acutely isolated WM, which is exceedingly robust and can be maintained in good working order for many hours devoid of GM (ie, no cell bodies or synapses). Several such preparations are in common use, including the corpus callosum slice, isolated spinal dorsal column and rodent optic nerve. This line of research has been highly profitable. We have learned that insulted WM loses excitability within minutes, that axons can suffer toxic Ca^{2+} overload due to influx of this ion and/or Ca^{2+} release from intracellular stores, and that

glutamate receptors participate in mediating WM injury.⁸ Until now, however, the preponderance of evidence favored the idea that glutamate receptors on oligodendrocytes were mainly, if not exclusively, to blame for the glutamate component of the injury (eg, Tekkok et al.^{2,9}).

Stys and colleagues bring credible new evidence that CNS axons themselves express glutamate receptors.^{5,6} Using an optical technique they have pioneered to monitor intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) in individual axons, they show that glutamate agonists cause slow increases in axonal $[Ca^{2+}]_i$. Pharmacological experiments and some immunohistochemical images support the conclusion that myelinated axons sport several subtypes of glutamate receptors under their myelin sheaths, including kainate and AMPA type receptors. When activated, these receptors admit Ca^{2+} , leading to a slight increase in axonal $[Ca^{2+}]_i$ that triggers further Ca^{2+} release from intracellular organelles. Some kainate type glutamate receptors appear to physically co-localize with L-type Ca^{2+} channels, while others associate with the enzyme that generates nitric oxide (NO), nitric oxide synthase. Activating these receptors leads to opening of the associated Ca^{2+} channels and NO production, both events contributing to more intracellular Ca^{2+} accumulation. Whether glutamate receptors on central myelinated axons serve a physiological purpose remains unaddressed, an obvious goal of future research. These findings do lend themselves to a revised theory about WM injury. The authors propose that glutamate released in WM under pathological conditions, for example ischemia or inflammation, would activate axonal glutamate receptors leading to direct axon injury via Ca^{2+} overload, as previously established.¹⁰ The work is elegant and pushes the techniques employed to their limits. It begs questions about glutamate source and how glutamate applied to the extracellular space manages to reach glutamate receptors buried under the myelin sheath, presumably a closed compartment for all practical purposes. More work will be necessary to determine the relative importance of axon vs. oligodendrocyte glutamate receptor activation in explaining irreversible loss of WM function during ischemia or traumatic insult. Alternatively, activation of these distinct receptor populations might be inextricably cooperative in producing WM injury.

These two papers, and the recent swell of others focused on WM, establish one formidable fact: This part of the CNS is far more complex than we imagined, based on its primary function of conveying electrical signals from one part of the brain to another. Intuitively, we expected no neurotransmitter receptors in this area, but now find evidence for glutamate receptors on every cell type tested. Their 'physiological' functions remain obscure, but they unequivocally can participate in harm. What Stys and his colleagues leave us to contemplate is how precisely all these glutamate receptors collaborate under injurious circumstances to excite axons to death.

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