Standing By: How Intact Neurons React to Axon Injury

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targeting instead non-vagal pontine nuclei involved in sickness (See conjectural model in Figure 1). Targeting this circuitry may, on one hand, eventually improve adherence to anorectic medications. In addition, since AP neurons appear to be preserved in area postrema syndrome patients, harnessing the relevant cell types may alleviate the burden of simultaneous nausea, vomiting, and hiccups.

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Standing By: How Intact Neurons React to Axon Injury

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Nerve injury affects the neurophysiology of severed and bystander axons. In this issue of *Neuron*, Hsu et al. demonstrate that this early effect is cell-autonomous and driven by dSarm, independently of its NADase activity otherwise required for axon degeneration. The authors show that axon injury signal spreads to intact neurons via glial cells.

In nervous system disorders or traumatic injuries, loss of connectivity is linked to degeneration of severed axons unable to further sustain signal conduction. This causes functional impairment of the circuit with devastating clinical consequences. However, it is necessary to reconsider this paradigm as it is becoming clearer that axon injury also affects the physiology of uninjured intact neurons (Greer et al., 2012), thereby altering neuronal function at the level of the entire circuit.

In this issue of *Neuron*, Hsu et al. (2020) describe the loss of physiological function in both severed and bystander axons. They perform injury of the L1 wing vein in *Drosophila*, a powerful model that allows rapid visualization of injury-induced events *in vivo*. Axonal transport is fundamental for correct neuronal functioning. In their study, authors combine the elegant techniques of mosaic analysis with a repressible cell marker (MARCM) and live imaging to track several types of axon cargoes at a single axon level, including synaptic vesicles and autophagosomes. They show that not only does the injury suppress axonal transport in damaged axons early after injury, but bystander intact neurons are also impacted.

The molecular cascade leading to explosive axon disassembly upon injury remains poorly understood. In this context, Wallerian degeneration stands as a standardized model that accounts for abrupt axonal loss upon injury (Conforti et al., 2014). Within a few hours after axotomy, axons undergo broad disassembly in the distal part, and within days, glial cells clear the debris. Remarkably, molecular actors of axon death response highlighted so far are evolutionarily conserved across species, including the fruit fly, the zebrafish, and the mouse. Importantly, the level of nicotinamide adenine dinucleotide (NAD+), a crucial source of energy for the axon, is depleted following injury. The conserved sterile α/Armadillo/Toll-interleukin receptor homology domain (dSarm/Sarm1) enzyme

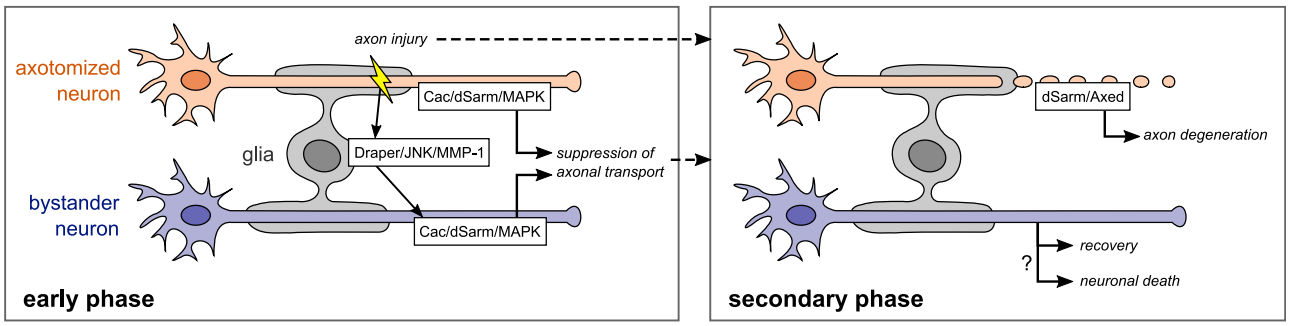

Figure 1. Distinct Roles of dSarm in the Cell-Autonomous Response of Injured and Bystander Neurons

During the early phase, both severed and bystander neurons have their physiology arrested in a molecular cascade implicating Cacophony (Cac), dSarm, and MAPK signaling. This cell-autonomous injury response is relayed by glial cells through Draper signaling. This early phase is opposed to a later phase, where dSarm is a key factor of NAD+ depletion leading to downstream Axed activation and explosive axon death and disassembly of injured neurons.

is a central mediator of axon degeneration (Osterloh et al., 2012), probably due to its NAD+ hydrolase activity (Llobet Rosell and Neukomm, 2019). Hsu et al. reveal that dSarm is required cell-autonomously for axonal transport suppression in both severed and bystander axons. Interestingly, dSarm NADase activity is not involved in this early phase of injury, while it is required for axon disassembly at a later phase. Furthermore, authors show that mutants null for Axed, a major effector of dSarm in axon degeneration, display no alteration of axonal transport. Rather, suppression of axonal transport in severed and uninjured axons depends on the voltage-gated calcium channel Cacophony and on MAPK signaling (Figure 1).

NAD+ depletion and accumulation of its substrate NMN, linked to Nmnat loss of activity, is a key mechanism underlying Wallerian degeneration (Llobet Rosell and Neukomm, 2019). Hsu et al. detect no effect in dNmnat mutants regarding axonal transport. However, surprisingly, dNmnat replenishment, through overexpression or expression of the in-frame fusion protein Wld^{S}, rescues axonal transport in injured and bystander neurons. This supports the hypothesis that dNmnat enhances dSarm activity early after injury, possibly via interaction with MAPK signaling. In perspective with the complex signaling involved in axon degeneration, these intriguing results reveal genetically distinct phases of the injury response with multi-faceted molecular players. These results also suggest the existence of feedback loops necessary to fine-tune their activity and cause the bystander effect.

One could expect that uninjured neurons would benefit from maintained connectivity with target cells and continuous energy supply. Yet, spared axons are still affected by the lesion. How are injury signals conveyed to intact axons? In their model, Hsu et al. observe that axons are individually wrapped by glial cells and hypothesize that injury signals travel via these. Using mutants null for Draper, a receptor of glial phagocytic function, they find that axonal transport is restored in bystander neurons. It remains to be determined whether glial cells relay the injury information from axotomized neurons or if they are themselves affected by the injury.

How the entire circuit is affected when only a subset of neurons is injured is a central question in the field of nervous system repair. Hsu et al. observe that the bystander effect is transient, possibly owing to the potential for spontaneous recovery in *Drosophila*, and in accordance with functional recovery observed in models of peripheral and central nervous system injuries (Greer et al., 2012). The potential for reversibility in bystander neurons is of particular interest in stroke. Following the primary acute ischemic injury, a secondary delayed wave of neuronal injury is relayed by axon-glia interactions (Hinman, 2014). Subsequent recovery or neuronal death opens a critical window for therapeutic intervention to preserve the intact circuit. In the case of the neuroinflammatory response, on the other hand, insult to the nervous system impacts the neurophysiology of bystander neurons, further affecting the functionality of the initially spared circuit in the long term. In this context, microglia become activated and relay injury signals through release of inflammatory cytokines toxic to intact neurons as well as to other glial cells (Liddelow et al., 2017).

It is important to note that glial cells' function is more complex than just a detrimental role for repair. In fact, microglia themselves have been shown to be necessary for neuron protection and preservation of correct functioning of the neuronal network after stroke (Szalay et al., 2016), although the molecular mechanism remains unclear. Moreover, glial cells are not the only relay of injury spreading to intact axons. Upon partial peripheral nerve injury, cytotoxic infiltrated immune cells mediate axon degeneration of neurons that are only partially damaged and that would show potential for functional recovery otherwise (Davies et al., 2019). Finally, synaptic connections themselves relay the injury information directly between axotomized neurons and intact neurons, which in turn elicit a pathophysiological response (Nagendran et al., 2017; Zhang et al., 2019). Thus, injury spreading to uninjured neurons takes place through various mechanisms that have to be considered in the design of therapies.

Altogether, Hsu et al.'s results point toward a dual role of dSarm corresponding to distinct phases of the neuronal injury response affecting axotomized and bystander neurons (Figure 1). These results highlight a time window during which neuronal physiology of the entire circuit is paused in response to injury, leading subsequently to functional—at least partial—recovery of bystander neurons on one hand and abrupt degeneration of

axotomized neurons on the other hand. Whether axon degeneration depends on this early phase of axonal transport suppression remains to be determined. This study offers new insight into the molecular events orchestrated by dSarm immediately after axonal injury. Deciphering the cell-autonomous molecular response of bystander neurons is crucial for the development of therapeutic strategies to preserve uninjured axons, limit injury spreading, and eventually promote functional repair of the nervous system.

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NMDA Receptors Singled out for Delayed Activation

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In this issue of *Neuron*, Amin et al. (2021) recorded single NMDA receptors in synaptic-like conditions to reveal how unreliable coupling between agonist binding and channel opening depends on structured linkers. After neurotransmitter binding, a complicated molecular "discussion" ensues, dividing fast synaptic events from delayed openings and failures.

Cartoons of glutamate receptors usually feature an ion channel domain and extracellular glutamate-binding domains. In the past decade, these doodles have been superseded by families of X-ray and cryo-EM structures (Greger and Mayer, 2019) that illustrate the complexity not only of the functional domains but also their coupling. NMDA receptors exhibit complex, slow activation that is triggered by binding of glycine to their NR1 subunits and glutamate to their NR2 subunits. But what applies the brakes to delay activation? The external domains sport large, malleable interfaces that are targets of both ions like zinc and protons and numerous drug-like compounds (Zhu and Paoletti, 2015). Dynamic gyrations of these domains either antagonize or boost activity, fingering them as the most obvious timewasters, through their changes in conformation. However, in this issue of *Neuron*, a new study (Amin et al., 2021) indicates that the three linkers that couple the external domains of each subunit to the ion channel throw much of the sand in the gears that make NMDA receptors so slow to activate. In this impressive piece of work, pinpoint engineered mutations slacken each linker in turn, disabling them, and reveal their specific roles in determining whether receptors activate quickly, slowly, or not at all.

Single-channel recordings of NMDA receptors date back to the 1980s, but measurements of the type in this paper (the "first latency to opening following a jump"; Aldrich et al., 1983) are exceedingly rare. It's the most advanced and challenging form of single-channel analysis, the main difficulty being to secure stable recordings from patches with exactly one channel. Few labs even attempt this method, and none have achieved the broad scope of the work presented here. The paper reports both equilibrium and non-equilibrium