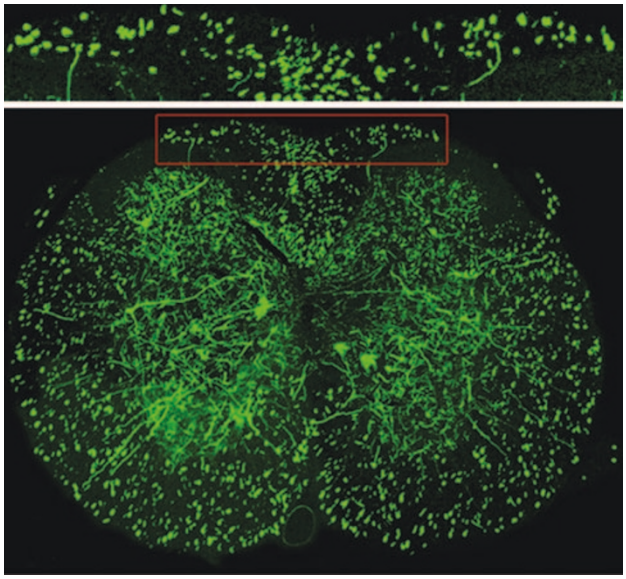
NEUROTECHNIQUE

Imaging spinal cord injury



Cross-section of the spinal cord of a transgenic mouse expressing green fluorescent protein with a subset of labelled axons. The red box (magnification ×2) delineates the superficial dorsal funiculus where sensory axons can be imaged *in vivo*. Reproduced, with permission, from *Nature Medicine*.

A technical report published recently in *Nature Medicine* shows that it is feasible to image injured axons in the spinal cord *in vivo*, disclosing new features of axonal degeneration and providing a powerful tool for evaluating therapies that might enhance regeneration.

Using transgenic mice that expressed green fluorescent protein in neurons of the dorsal root ganglia (DRG), Kerschensteiner *et al.* succeeded in visualizing DRG axons over several spinal segments with wide-field microscopy. They then lesioned a bundle of axons containing a single labelled fibre, and followed the fate of its proximal and distal ends for several days.

They found that the axon ends were stable for the first 10–20 min after the lesion, but then underwent a sudden fragmentation, a process that the authors termed 'acute axonal degeneration' (AAD). This dieback process lasted less than 5 min, but accounted for nearly 90% of the axonal loss that was observed 4 h after injury. Moreover, if AAD of the proximal axon spread beyond a branch point, it also resulted in disconnection of the unlesioned branch from the soma.

By 30 h after injury, both the proximal and distal axons had died back symmetrically; the degeneration extended 300 μm from the lesion site in both directions. By contrast, at later time points, the distal axon experienced the well-known process of Wallerian degeneration, whereas the proximal axon remained stable and, in some cases, even attempted to regrow. However, these attempts to regenerate were remarkably inefficient; the regenerating axons seemed to lack directional information. The authors found no axon that managed to grow back to the lesion site, even though they grew long distances (as long as 1 mm 2 weeks after the lesion). Instead, fibres grew laterally or even in a 'U-turn' trajectory.

It is interesting to compare this erratic behaviour with the ability of peripheral axons to grow in a straight trajectory after a lesion, following a route close to their original path. The inability of central axons to navigate in the proper direction might account, at least in part, for their limited success in extending past the lesion site.

AXON GUIDANCE

Channel surprise

The growth cones of developing axons navigate through a complex environment, and along the way they read molecular signals that tell them where and when to stop, go and turn. It is possible to simulate growth cone guidance in a dish, by culturing neurons and exposing their growth cones to gradients of attractive or repulsive molecules delivered by a micropipette. The growth cones respond by turning towards or away from the pipette.

Using this deceptively simple technique, Mu-ming Poo and colleagues have assembled a substantial body of work to show that the turning response to an attractive signal, such as brain-derived neurotrophic factor (BDNF) or netrin, requires a minimum level of intracellular cyclic nucleotides, and depends on the formation of calcium gradients across the growth cone. Indeed, the attractive response can be switched to repulsion if either cyclic AMP activity or calcium influx are low. Both attractive and repulsive responses require calcium influx. But how does calcium enter the growth cone? Earlier work has shown that L-type voltage-dependent calcium channels account for part of the influx, and two papers published in *Nature* now show that transient receptor potential canonical (TRPC) channels must be activated before the L-type channels can kick in.

Working with *Xenopus* spinal neurons, Wang and Poo found that the depolarization caused by the attractive cue netrin was completely abolished in the presence of a TRPC channel inhibitor, whereas other inhibitors were only partially effective. Knockdown of the *Xenopus* TRPC1 channel by morpholino antisense abrogated calcium influx and attraction in response to a netrin gradient. This finding, together with pharmacological inhibitor experiments, led the authors to conclude that TRPC1-mediated depolarization and calcium influx are prerequisites for the subsequent activation of L-type channels.

Li and colleagues concomitantly report their study of rat cerebellar granule neurons. A series of inhibitors of voltage-gated calcium and sodium channels did not affect growth cone attraction to gradients of BDNF or glutamate. Meanwhile, a TRPC channel inhibitor abrogated attraction to BDNF without affecting attraction to glutamate.

The authors found that cerebellar granule neurons express three TRPC channels. Inhibition of TRPC3 or TRPC6, by small interfering RNAs or dominant-interfering constructs, abolished growth cone attraction to BDNF (but not to glutamate), whereas knockdown of TRPC1 had no effect. Further pharmacological inhibitor experiments confirmed that, as in *Xenopus* neurons, attraction to BDNF also required the BDNF receptor TrkB and phospholipase Cγ (PLCγ). An agonist of PLCγ alone was sufficient to attract granule neuron growth cones, depending on extracellular calcium and

Despite their different time courses, AAD and Wallerian degeneration might share similar molecular mechanisms. The authors found that in 'Wallerian-degeneration slow' mice, which show delayed Wallerian degeneration, AAD was largely absent. Moreover, calpain, which is known to participate in Wallerian degeneration, also seems to be a mediator of AAD, as is shown by the ability of calpain inhibitors to prevent it *in vivo*. Additional similarities and differences between AAD and Wallerian degeneration might now be established using this technique.

Beyond the description of the degeneration process, this method can also be used to monitor the efficacy of interventions aimed at preventing degeneration or promoting regeneration, as exemplified by the use of calpain inhibitors in this report. Moreover, as the response of axons to damage is relevant not only to spinal cord injury but also to conditions such as multiple sclerosis and amyotrophic lateral sclerosis, this technique might be used to provide new insights into these diseases.

Juan Carlos López, Chief Editor,
Nature Medicine

References and links

ORIGINAL RESEARCH PAPER Kerschensteiner, M. *et al.* *In vivo* imaging of axonal degeneration and regeneration in the injured spinal cord. *Nature Med.* 10 April 2005 (10.1038/nm1229)

TRPC channels. The authors propose a model whereby activation of TrkB by BDNF triggers, through PLCγ, the release of calcium from intracellular stores, which provides a sufficient increase in calcium concentrations to activate TRPC channels, allowing an influx of extracellular calcium, depolarization and turning.

TRPCs are part of a large family of TRP channels. TRPs are widely expressed, and have been implicated in many, especially sensory, functions. These two *Nature* reports indicate that TRPCs have an essential role in axon pathfinding. This exciting idea now awaits confirmation *in vivo*.

Annette Markus, Associate Editor,
Nature Neuroscience

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FURTHER READING Henley, J. & Poo, M.-m. Guiding neuronal growth cones using Ca^{2+} signals. *Trends Cell Biol.* **14**, 320–330 (2004)
WEB SITES
Poo's laboratory: http://mcb.berkeley.edu/labs/poo/
Institute of Neuroscience, Shanghai: http://www.ion.ac.cn/

Image courtesy of Lawrence Keogh

DEVELOPMENT

NF-κB branches out

The widely expressed transcription factor nuclear factor-κB (NF-κB) has a well-characterized role in many cellular processes, including inflammation, immune- and stress-related responses, and the regulation of cell survival and proliferation, in all tissues. Recent studies have also highlighted several neural-specific functions for NF-κB, including an involvement in learning and memory, although the mechanisms that underlie these processes are not well understood. Now, however, Gutierrez and colleagues, reporting in *Development*, have uncovered a new and unexpected role for NF-κB in promoting the growth and branching of neural processes in the nervous system during development.

To investigate the function of NF-κB in the nervous system, Gutierrez and colleagues studied the activation of NF-κB in two well-characterized populations of developing PNS and CNS neurons: the sensory neurons of the embryonic and neonatal nodose ganglion, and layer 2 pyramidal neurons in the postnatal somatosensory cortex of mice. In both populations, preventing NF-κB activation or its transcriptional activity substantially reduced the overall span, total neurite length and total branch number of the neurite arbours. However, there was no effect on neuronal survival, which indicates that these changes were not the consequence of a general detrimental effect on neuronal viability, nor could they be explained by a global reduction in cellular metabolism, as there was no effect on soma size.

Importantly, this effect of NF-κB on the promotion of neurite growth was specific to a critical phase of neuronal development. In the nodose ganglion cells, it took place following the phase of naturally occurring neuronal death, when the axons of the remaining neurons elaborate and modify their terminal arborizations; and in the neurons of the somatosensory cortex it occured when the dendritic arbours grow rapidly and establish functional connections.

It remains to be determined whether NF-κB exerts its effects on neurite growth directly or through its action on the expression of other genes that are involved in the growth of neural processes. Nevertheless, this new role for NF-κB sheds light on the molecular mechanisms that underlie the growth and branching of axonal and dendritic processes, which are crucial for functional development and plasticity in the nervous system. Moreover, this finding might explain the increasing evidence that NF-κB is linked to learning and memory, as establishing and refining neuronal processes is thought to be important for these functions.

Alison Rowan

References and links

ORIGINAL RESEARCH PAPER Gutierrez, H., Hale, V. A., Dolcet, X. & Davies, A. NF-κB signalling regulates the growth of neural processes in the developing PNS and CNS. *Development* **132**, 1713–1726 (2005)