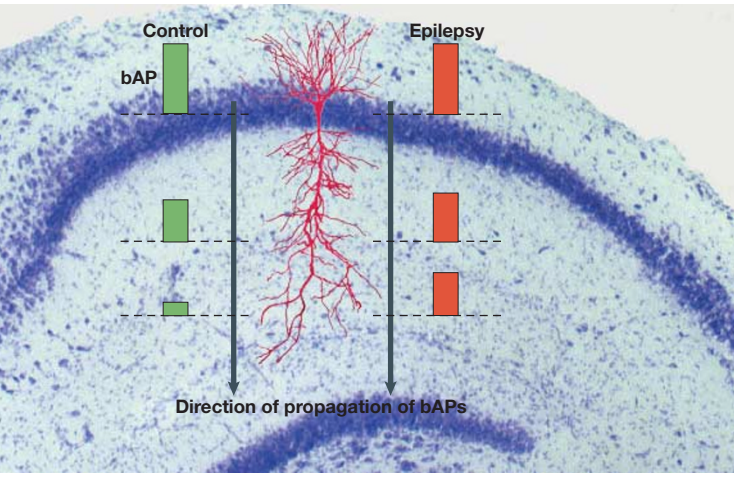
NEUROLOGICAL DISORDERS

The current view of epilepsy



Reconstruction of a CA1 pyramidal cell superimposed on its environment. Action potentials are generated in the perisomatic region, and their amplitude reaches 100 mV. They can backpropagate in the dendrites, but their amplitude decreases with distance from the soma in control tissue (green bars). In the distal dendrites, the backpropagated axon potential (bAP) amplitude only reaches 2–3 mV, because the density of potassium channels increases with the distance from the soma, thereby reducing the amplitude of bAPs as they backpropagate. In epileptic tissue (red bars), this control by potassium channels is decreased and the amplitude of bAPs in distal dendrites remains around 40 mV. Image courtesy of C. Bernard, INSERM.

When an epileptic seizure occurs in a neuronal circuit, the likelihood of subsequent seizures in that circuit is often increased. In some cases at least, this seems to be accomplished by the formation, through axon sprouting, of new excitatory connections between neurons that fired synchronously during the seizure. However, in *Science*, Bernard *et al.* now report on a 'wireless' mechanism for seizure facilitation, in which dendritic excitability is increased through the loss of an inhibitory potassium current.

When an action potential is generated in the cell body of a neuron, the signal is predominantly transmitted down the axon, but some of the activity can be reflected, or backpropagated, into the dendritic tree. Normally, these backpropagated action potentials (bAPs) are dissipated by an outward potassium current, which is known as the A current because it is generated by the opening of A-type potassium channels in the dendrites.

To investigate the effects of seizure activity on the A current, Bernard *et al.* generated a rat model of temporal lobe epilepsy. They treated the rats with the acetylcholine-receptor agonist pilocarpine to trigger a prolonged seizure, which led to the development of chronic spontaneous seizures several weeks later. The authors took recordings from the dendrites of CA1 pyramidal neurons in hippocampal slices from the epileptic rats, and they observed unusually high bAP amplitudes at a distance of more than 250 m from the soma (the bAP amplitude usually diminishes markedly with distance from the soma). Although the authors did not measure the A current directly, the increased bAP amplitude was taken to indicate a reduction in this current.

The authors found that both the synthesis and function of A-type

AXON GUIDANCE

Translating the cues

The control of growth-cone behaviour by axon guidance cues involves regulating local protein synthesis and axonal transport; however, the precise molecular mechanism is still unknown. Reporting in the *Journal of Neuroscience*, Li and colleagues found that semaphorin3A (SEMA3A) induces axonal transport by activating a translation-initiation factor at the growth cone and that the process depends on the tyrosine kinase FYN and the cyclin-dependent kinase 5 (CDK5).

SEMA3A is a secreted guidance cue that is strongly repulsive to axons from dorsal root ganglia (DRG) and sympathetic neurons. Signalling by SEMA3A requires the activity of both FYN and CDK5, as inhibitors of either molecule can block the collapsing effect of SEMA3A on growth cones. In this study, the authors assessed whether SEMA3A-elicited axonal transport in cultured DRG neurons also requires FYN and CDK5 by using a video-enhanced contrast differential interference video camera system. They found that inhibitors of either FYN or CDK5 abolished the ability of SEMA3A to induce anterograde and retrograde axonal transport. Similarly, SEMA3A-induced axonal transport is also attenuated in DRG neurons from mice deficient in either *Fyn* or *p35*, an activator of CDK5.

The authors then measured SEMA3A-elicited axonal transport in the presence of inhibitors of either protein synthesis or degradation. Inhibition of protein synthesis suppressed axonal transport and SEMA3A-induced growth-cone collapse, but inhibition of degradation did not.

The signalling cascades that stimulate axonal transport can occur either locally, at the growth cone, or globally, involving the cell body. To differentiate between these possibilities, the authors performed similar assays using neurites that were severed from the soma. They showed that growth cones from isolated neurites also collapsed in response to SEMA3A and that acceleration of axonal transport was similar to that in intact neurons. Furthermore, inhibitors of FYN, CDK5 and protein synthesis could also block SEMA3A-induced axonal transport in isolated neurites. Therefore, activation of local signalling cascades and local protein synthesis is responsible for SEMA3A-induced axonal transport.

The authors then attempted to pinpoint which translation factors are involved. They found that eukaryotic translation-initiation factor 4E (eIF-4E) at the growth cone was phosphorylated in response to SEMA3A — an effect that was diminished by inhibitors of either FYN or CDK5. Similarly, SEMA3A-induced phosphorylation of eIF-4E was also compromised in DRG neurons from *Fyn*$^{-/-}$ and *p35*$^{-/-}$ mice.

The study suggests an interesting link between the functions of FYN and CDK5 and local activation of a translation-initiation factor at the growth cone in response to guidance cues. Further analysis is necessary to delineate the signalling mechanisms and to identify newly synthesized target proteins that are responsible for initiating downstream molecular events in axon guidance and neural plasticity.

Jane Qiu

References and links

ORIGINAL RESEARCH PAPER Li, C. *et al.* Correlation between semaphorin3A-induced facilitation of axonal transport and local activation of a translation initiation factor eukaryotic translation initiation factor 4E. *J. Neurosci.* **24**, 6161–6170 (2004)

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channels was affected by the seizures. In the epileptic animals, transcription of the KV4.2-channel gene was downregulated, and the activity of many of the remaining channels was decreased through protein kinase C-mediated phosphorylation.

Many studies have implicated inherited ion-channel defects in epilepsy, and this study illustrates that channelopathies can also be acquired as a result of seizure activity. If similar phenomena are shown to underlie seizure facilitation in humans, new anti-epileptic drug targets might be identified on the basis of the molecular mechanisms that modulate dendritic excitability.

Heather Wood

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WEB SITE

Encyclopedia of Life Sciences:
http://www.els.net/
epilepsy

NEUROLOGICAL DISORDERS

A new role for *spastin*

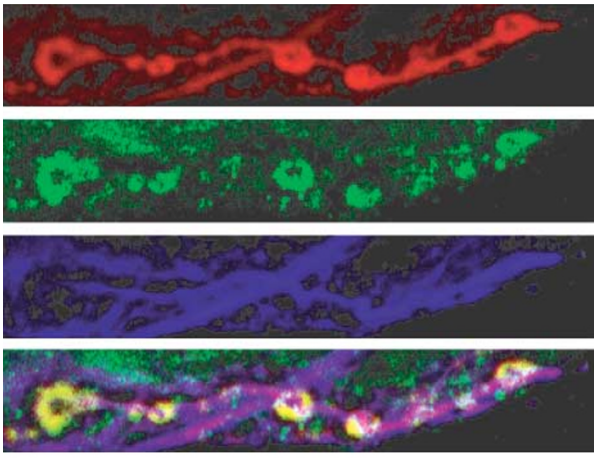
Hereditary spastic paraplegia is a devastating motor disorder that causes spastic weakness of the lower limbs and eventual axonal degeneration. More than 40% of all cases are associated with mutations in one gene, *spastin*, but little is known about how such mutations cause the disease. Reporting in *Current Biology*, Trotta *et al.* found that *spastin* mutations might lead to defects in neurotransmission by affecting microtubule functions.

Spastin is an ATPase that contains a microtubule-interacting domain. Therefore, the authors asked whether microtubule structure or function might be affected by abnormal *spastin* expression. They studied this in the *Drosophila melanogaster* neuromuscular junction (NMJ) synapse, which has been widely used to assay functions of the microtubule cytoskeleton and to model its role in inherited neurological diseases.

Spastin is expressed at high levels in the nervous system of both mammals and *D. melanogaster*, but its subcellular localization has not been characterised. Trotta *et al.* found that the protein is highly enriched in axons and synaptic connections. They showed that spastin co-localizes with a synaptic vesicle protein, synaptotagmin, indicating that spastin is expressed in the synaptic vesicle pool domain of the presynaptic bouton.

The authors showed that knockdown of ubiquitous *spastin* expression by RNA interference causes lethality. When they knocked down *spastin* expression specifically in the nervous system, the animals had very poor coordination and locomotor abilities. Interestingly, overexpression of *spastin*, either ubiquitously or specifically in the nervous system, was also lethal to embryos or early larvae. The authors suggest that a correct dose of *spastin* expression is crucial for normal development.

Spastin interacts with microtubules and prevents their assembly *in vitro*. The authors assessed the effects of altered *spastin* expression *in vivo* on microtubule assembly and synaptic transmission. They found that, at the NMJ presynaptic terminal, *spastin* knockdown in the neuron led to an accumulation of acetylated α-tubulin, the post-translationally modified form of tubulin that occurs only in structurally stable microtubules. Conversely, neuron-specific overexpression of *spastin* caused a reduction in stabilized tubulin and, often, the stabilized tubulin network was no longer detectable.



Confocal micrograph of a *Drosophila melanogaster* neuromuscular junction. Signals represent immunoreactivity for different antibodies. Red, anti-horseradish peroxidase labelling neuronal membranes; green, anti-D-spastin; blue, anti-acetylated tubulin, which detects stable and long-lived microtubule filaments. Note that regions where D-spastin is enriched also appear to be regions where stable microtubules are excluded. Image courtesy of K. Broadie, Vanderbilt University, USA.

The authors showed that these effects on microtubule assembly correlated with changes in synaptic transmission. When they stimulated the motor nerve and measured glutamate-gated synaptic currents in the voltage-clamped muscle, they found that loss of *spastin* expression resulted in an increase in current amplitude, whereas *spastin* overexpression had the opposite effect. These effects could be reversed by pharmacological agents that affect microtubule stability. Normal functions were restored in *spastin* knockdown flies by nocodazole, which disassembles microtubules, and in flies overexpressing *spastin* by taxol, which stabilizes tubulin monomers. In both cases synaptic transmission was indistinguishable from that in normal animals.

The study shows that *spastin* is enriched at the synapse and controls synaptic transmission by regulating microtubule assembly. Trotta *et al.* conclude that it is likely that defects in microtubule stability are the primary cause of hereditary spastic paraplegia. This mechanistic insight has significant implications in designing therapeutic strategies to treat the illness.

Jane Qiu

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ORIGINAL RESEARCH PAPER Trotta, N. *et al.* The hereditary spastic paraplegia gene, *spastin*, regulates microtubule stability to modulate synaptic structure and function. *Curr. Biol.* **14**, 1135–1147 (2004)

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