This Week in The Journal

Loss of Neurofibromin Causes Hypersensitivity in *Drosophila*

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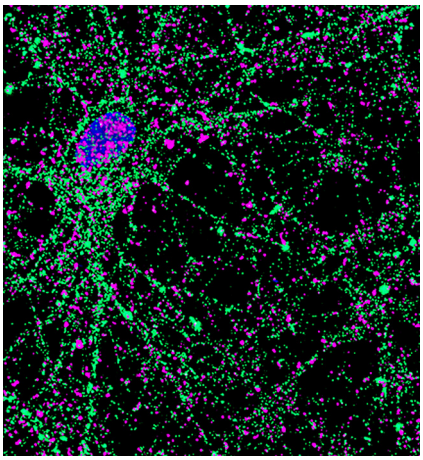
(see pages 9450–9472)

Pathological variants of the gene encoding neurofibromin (NF1) cause neurofibromatosis, a disorder characterized by the development of tumors in both the PNS and CNS, often accompanied by cognitive, motor, and social deficits. By catalyzing GTP hydrolysis, NF1 switches off signaling cascades initiated by Ras-family small GTPases. It also regulates signaling through cAMP, and it may regulate several other proteins through direct interactions. Work in animal models has attributed some of the cognitive deficits resulting from the loss of NF1 function to enhanced GABAergic inhibition or reduced dopaminergic signaling. Dyson et al. now suggest that dysfunction of cholinergic neurons underlies mechanical hypersensitivity in NF1-deficient *Drosophila* larvae.

When the *Drosophila* NF1 homolog was knocked out, larvae generated corkscrewing movements, normally evoked by noxious stimuli, in response to light mechanical stimuli. This abnormal behavior was accompanied by changes in glutamatergic transmission at the neuromuscular junction. Specifically, the loss of NF1 reduced release probability and the number of vesicles released per action potential in motor neurons. It thus reduced the current evoked in muscle cells in response to motor nerve stimulation. The reduction in evoked release may have been a consequence of increased spontaneous vesicle release, as suggested by an increase in the frequency and amplitude of miniature excitatory junction potentials. In addition, NF1 knockout led to increased burst activity in motor neurons, suggesting it increased neuronal excitability. Surprisingly, a similar phenotype—increased spiking in motor nerves and hypersensitivity to mechanical stimuli—was produced by knocking down NF1 selectively in cholinergic neurons, which comprise ~80% of neurons in the *Drosophila* CNS, but not by knocking down NF1 selectively in peripheral sensory neurons, dopaminergic neurons, or glutamatergic neurons (which include motor neurons in *Drosophila*). Finally, knocking down Ras proteins fully rescued neural hyperexcitability and mechanical hypersensitivity in NF1-deficient larvae.

These results suggest that the loss of NF1 increases RAS-dependent regulation of activity in excitatory cholinergic neurons in *Drosophila* larvae, leading to increased excitability of glutamatergic motor neurons and hypersensitivity to mechanical stimuli. Future experiments should determine which cholinergic neurons are involved in this response and how Ras regulates their activity.



Hyperphosphorylated α-synuclein aggregates (magenta) accumulate near postsynaptic densities (green) in cultured cortical neurons exposed to PFFs. See Hallam RD et al. for details.

Synuclein Fibrils Induce S-Nitrosylation of MAP1A

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(see pages 9473–9487)

Lewy bodies—intraneuronal inclusions of aggregated α-synuclein protein, are defining features of Parkinson's disease (PD) and dementia with Lewy bodies (DLB). In healthy neurons, α-synuclein is located predominantly in presynaptic terminals, where it is thought to regulate synaptic vesicle release. Aggregation of α-synuclein in PD and DLB leads to loss of synapses followed by neuron degeneration; the underlying cellular and molecular mechanisms are poorly understood, however. To address this question, Hallam, Buchner-Duby, et al. exposed cultures of rat cortical neurons to preformed fibrils (PFFs) of human α-synuclein, a treatment that was previously shown to induce aggregation of endogenous α-synuclein in neurons.

Treating cortical cultures with PFFs caused total levels, phosphorylation, and aggregation of endogenous α-synuclein to increase within 24 h of treatment, and levels continued to increase for at least 7 d. Notably, levels of phosphorylated α-synuclein were increased in both presynaptic and postsynaptic compartments. In addition, PFF treatment decreased spontaneous neuronal activity and activity evoked by glutamate or NMDA (but not AMPA) stimulation. Finally, PFF treatment reduced the density of mushroom-shaped (mature) dendritic spines.

Consistent with previous work, PFF treatment increased neuronal accumulation of nitric oxide (NO), which can lead to S-nitrosylation of many proteins. Bioinformatics screening identified four proteins that both interact with NMDA receptors and are subject to S-nitrosylation. But only one of these proteins, MAP1A, showed increased S-nitrosylation in cortical cultures treated with PFF. Importantly, blocking NO synthase (NOS) prevented S-nitrosylation of MAP1A, loss of dendritic spines, reduction in glutamate-evoked activity, and inhibition of network activity in PFF-treated cortical cultures.

These results suggest that aggregates of α-synuclein impair synaptic function in part by increasing the activity of NOS, leading to S-nitrosylation of MAP1A. Previous work showed that MAP1A helps maintain NMDA receptor function, possibly by helping to tether the receptors to the underlying cytoskeleton. S-nitrosylation appears to disrupt this role of MAP1A, resulting in reduced NMDA receptor activity, and thus loss of dendritic spines. Importantly, NOS levels are increased in brains of PD patients and in animal models of PD, indicating that this pathway may play a key role in PD and DLB pathology.

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