Restoring neuron connections

A synthetic peptide promotes functional recovery in neurodegeneration and spinal cord injury models.

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The structural and functional integrity of synapses is crucial for neuronal circuit function and for the ability of animals to respond and adapt to their environment. However, synapses become dysfunctional or are lost in developmental and neurodegenerative disorders, and after brain and spinal cord injury (SCI). Deficiency in synapse integrity contributes to cognitive impairment and motor disability. To restore damaged neuronal circuits after SCI, antibody therapy against molecules such as Nogo, which inhibits axon growth, are in clinical trials (*1*). For other conditions such as Alzheimer's disease (AD), blockade of the pathogenic molecule amyloid-β or its downstream effects are being tested as potential therapies (*2*). To develop effective approaches for restoring impaired synapses, careful consideration should be given to the diversity of synaptogenic factors. To overcome this, on page 1074 of this issue, Suzuki *et al.* (*3*) report a structurally guided synthetic peptide to restore synaptic integrity of damaged and impaired circuits in mice.

The formation of synapses requires the combinatorial function of several molecules, including extracellular secreted molecules [Wnts, fibroblast growth factors (FGFs), and semaphorins], transmembrane proteins [neuroligin (NLGN), neurexin (NRX), ephrin receptors, and synaptic cell adhesion molecule (SynCAM)], and also components of the extracellular matrix present at the synaptic cleft, such as extracellular scaffolding proteins (ESPs) (*4*, *5*). ESPs, which include cerebellin (CBLN1) and neuronal pentraxin 1 (NP1) (*6*), form bridges across the synaptic cleft, allowing the coordinated assembly of the presynaptic terminal that contains the machinery for neurotransmitter release and the postsynaptic side with neurotransmitter receptors, ion channels, and signaling molecules. These synaptic organizers could be ideal molecules to restore defective synaptic connections. However, the diversity of these molecules in different brain regions poses a challenge for designing strategies to repair damaged neuronal circuits.

Suzuki *et al.* designed a molecule that could promote the formation of synapses in various brain regions by taking advantage of the structure of two widespread ESPs, CBLN1 and NP1 (see the figure). The synthetic peptide, CPTX, contains a key binding domain from each of these proteins: the amino-terminal cysteine-rich region (CRR) of CBLN1 and the pentraxin (PTX) domain of NP1 (NP1-PTX). The CRR binds specific ectodomains of NRX1b, a protein present at most presynaptic terminals, whereas NP1-PTX binds to the extracellular regions of postsynaptic glutamate receptors. CPTX therefore forms a molecular bridge between the pre- and postsynaptic sides, promoting the assembly of glutamatergic synapses. Suzuki *et al*. evaluated the effectiveness of CPTX in promoting synapse formation in vivo. CPTX injection into the adult hippocampus of wild-type mice promotes the formation of functional glutamatergic synapses 3 days after injection. To address the potential beneficial effect of CPTX on synaptic recovery, the authors injected CPTX in mice lacking the glutamate receptor delta-2 subunit (*GluD2*) or *Cbln1* genes, which exhibit defects in

synapse number, synaptic function, and ataxia (*7*, *8*). Three days after injection of CPTX into the cerebellum of these adult mice, synaptic connectivity was evaluated by using cellular, electrophysiological, and behavioral approaches. In both models, CPTX increased the number of connections between Purkinje cell (PC) neurons and the parallel fibers (PF) axons of granule cell neurons, the postsynaptic partners of PCs. CPTX also restored the functional connectivity between PFs with PCs and improved motor coordination. Thus, CPTX promotes structural and functional recovery of neuronal circuits in cerebellar dysfunction in mice.

To further test the utility of CPTX for therapeutic use, the authors investigated its effect on a model of neurodegeneration and also on a model of SCI. They used 5xFAD mice, an established mouse model of early familial AD. These mice exhibit synapse degeneration and defects in both long-term potentiation [(LTP), which reflects persistent strengthening of synapses], and hippocampal-mediated memory (*9*). Injection of CPTX into the hippocampus of 15- to 18-month-old mice—the time when synaptic connectivity is severely impaired—completely restored the number of postsynaptic structures (dendritic spines), LTP, and hippocampal-mediated memory. The effectiveness of CPTX was also tested in a mouse model of SCI where injury was caused by hemisection at thoracic level, a procedure that damages the motor nerve tracks that innervate skeletal muscles (*10*). Injection of CPTX at the time of the hemisection or a week later resulted in substantial functional recovery. Suzuki *et al*. also induced injury by contusion or compression, which is considered the best model for SCI in humans. Injection of CPTX into the spinal cord induces functional recovery even when tested 8 weeks after the injury. These exciting findings demonstrate that CPTX induces functional recovery after a single injection and its effect persists for several days or weeks.

Analyses of the functional recovery induced by CPTX reveal two interesting findings. One is that CPTX induces functional recovery even when the protein is cleared from the tissue (after 3 days). These findings suggest that CPTX triggers a recovery cascade that persists beyond its presence in the tissue. In addition, the long-term effects of CPTX vary in different brain regions and/or the nature of the injury. In the cerebellum, CPTX injection in *Cbln1* mutant mice induces a transient functional recovery that lasts only a few days. By contrast, CPTX injection into the spinal cord induces functional recovery that lasts several weeks. Further studies are needed to elucidate the molecular mechanisms by which CPTX regulates synapse recovery in different regions of the central nervous system or mode of injury.

The study of Suzuki *et al.* demonstrates that CPTX is a powerful molecule that restores synaptic connectivity and behavioral function in different brain regions and pathological conditions. The biggest challenge now is translating these promising and exciting results into medical applications. Several aspects need to be considered. CPTX could have side effects—for example, by inducing an imbalance between excitatory and inhibitory synapses. Although this seems unlikely as the animals do not exhibit signs of increased excitability, further studies are needed to test potential adverse effects. The mode of delivery of the synthetic peptide is important, as direct injection into the brain is an invasive procedure. Alternatively, intravenous injection could be used, but then the peptide (or analogs) needs to cross the blood–brain barrier to access the brain. Given the size and structure of CPTX, it is unlikely to do so. In the future, CPTX or newly designed analogs that can access the brain could provide an

effective treatment for neurodevelopmental disorders, neurodegenerative conditions, and nerve injury.

REFERENCES AND NOTES

1. K. Kucher et al., Neurorehabil. Neural Repair 32, 578 (2018).
2. Alzforum (2020); www.alzforum.org/therapeutics.
3. K. Suzuki et al., Science 369, eabb4853 (2020).
4. T. C. Südhof, Neuron 100, 276 (2018).
5. V. Budnik, P. C. Salinas, Curr. Opin. Neurobiol. 53, 90 (2011).
6. M. Yuzaki, Annu. Rev. Physiol. 80, 243 (2018).
7. A. Ito-Ishida et al., J. Neurosci. 28, 5920 (2008).
8. K. Matsuda et al., Science 328, 363 (2010).
9. H. Oakley et al., J. Neurosci. 26, 10129 (2006).
10. G. Courtine, M. V. Sofroniew, Nat. Med. 25, 898 (2019).

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FIGURE: