



Unique transsynaptic complexes enable long-term synaptic plasticity in a synapse-specific manner

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Long-term potentiation (LTP) of synaptic transmission, possibly one of the most studied cellular phenomena in neuroscience, refers to the long-lasting strengthening of a synaptic connection typically induced by brief repetitive activity. Much of the excitement about this phenomenon relies on the early idea that LTP could be a cellular correlate of some forms of learning and memory. Despite intense research on LTP since its discovery five decades ago (>17,000 publications listed in PubMed), important knowledge gaps remain regarding its molecular mechanisms and functional relevance. Growing evidence indicates that transsynaptic adhesion complexes known to play key roles in synapse development are also implicated in LTP at mature synapses, although the precise mechanism is poorly understood. A recent study published in PNAS has identified a transsynaptic protein–protein interaction that is essential for LTP but not for basal evoked synaptic transmission (1) (Fig. 1).

LTP was first reported in the dentate gyrus, the main entry area of the hippocampus, at the excitatory synapse established between pyramidal neurons in the entorhinal cortex (EC) and granule cells (GC) (2), but it was later identified at several other synapses in vertebrates and invertebrates. LTP is not a unitary phenomenon. Several mechanistically distinct forms of long-term synaptic plasticity at both excitatory and inhibitory synapses have been reported, and it is unlikely that learning relies on a single form of activity-dependent synaptic plasticity. However, most studies, by far, have focused on a “classical” form of excitatory LTP (aka Hebbian LTP) like the one originally identified at EC–GC synapses. There, calcium influx via transient activation of N-methyl-D-aspartate receptors (NMDARs) by the neurotransmitter glutamate triggers a long-lasting synaptic strengthening that is most likely due to the postsynaptic recruitment of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA). LTP is accompanied by other changes such as an increase in synapse size and is regulated by numerous molecules. A recent addition to the long list of molecules implicated in LTP includes transsynaptic adhesion complexes. For example, neuroligin-1 and LRRTMs, two cell adhesion molecules which are postsynaptic receptors for presynaptic neurexins (Nrxn), are required for LTP induction at CA3 to CA1 pyramidal cell synapses (3), but whether these transsynaptic complexes are also required at other excitatory synapses remains unclear. Other postsynaptic receptors for presynaptic Nrxn, which have also been implicated in synaptic function, include the glutamate receptors GluD1 and GluD2. GluD1 is widely expressed in the brain and is required for normal AMPAR- and NMDAR-mediated synaptic transmission in the hippocampus (4, 5), whereas GluD2 is expressed in Purkinje cells and is required for long-term depression (LTD) in the cerebellum (6). Thus, consistent with the remarkable molecular diversity of synapses

(7), transsynaptic complexes of distinct molecular composition can control basal synaptic transmission and long-term plasticity.

Cerebellins (Cbln) are secreted adaptor molecules that connect presynaptic neurexins to postsynaptic receptors, thereby forming transsynaptic Nrxn–Cbln–receptor complexes. Mammals contain four *Cbln* genes (*Cbln1* to *Cbln4* in mice) that are widely, albeit unevenly, expressed in the brain. Using RNA fluorescent in situ hybridization, Liakath-Ali et al. (1) find that the EC expresses high levels of *Cbln4*. *Cbln4* is also expressed in hippocampal interneurons but not in principal cells (i.e., GCs and pyramidal cells), while neurexins (*Nrxn1*, *Nrxn2*, and *Nrxn3*) are highly expressed in the EC. The EC projects to GCs via the medial and lateral perforant paths—MPP and LPP, respectively. Remarkably, presynaptic deletion of *Cbln4* using an acute conditional knockout (cKO) approach, that is, by injecting a Cre-viral vector in the EC, abolished LTP at both MPP–GC and LPP–GC synapses but had no effect on basal evoked synaptic transmission. This finding represents evidence for *Cbln4* playing a role in synaptic plasticity.

Next, the authors sought to determine the *Cbln4* target and identified neogenin 1 (Neo1), a multifunctional transmembrane receptor, as the postsynaptic receptor for *Cbln4* (1). Neo1 is highly expressed in GC dendrites (8) and is required for LTP in the basolateral amygdala (9). Using a cell aggregation assay in HEK cells, they found that Neo1 binds *Cbln4*/Nrxn1 complexes, although the identity of the specific Nrxn isoform(s) involved in EC–GC LTP was not determined. In addition, postsynaptic deletion of *Neo1* (again, using viral vectors in the mature brain), severely impaired EC–GC LTP but had no effect on basal synaptic transmission, indicating that postsynaptic deletion of *Neo1* phenocopied presynaptic deletion of *Cbln4*. Together, these findings strongly suggest that secreted *Cbln4* promotes the formation of transsynaptic complexes between presynaptic Nrxn and postsynaptic receptor Neo1, which renders EC–GC synapses competent for LTP.

The transsynaptic Nrxn–Cbln4–Neo1 complex requirement for LTP at MPP–GC and LPP–GC synapses resembles

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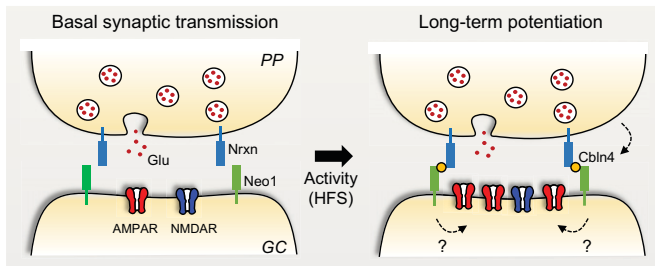


Fig. 1. Transsynaptic signaling mediates LTP at the glutamatergic perforant path (PP) to GC synapse of the mouse dentate gyrus. High-frequency stimulation (HFS) of PP inputs releases the Nrxn-ligand Cbln4 which binds postsynaptic Neo1. The Nrxn-Cbln4-Neo1 transcellular complex enables LTP whose expression mechanism involves a postsynaptic enhancement of AMPARs but not NMDARs. The signaling mechanism downstream of Neo1 that mediates LTP is unknown.

the transsynaptic interaction of presynaptic Nrxns with postsynaptic neuroligin-1 and LRRTMs which is required for LTP in the CA1 area of the hippocampus (3, 10). Given that Cbln4 is not detected in the CA1 area (1), another secreted Cbln is likely involved in this area. Unlike *Neo1* cKO in the mature dentate gyrus (1), *Neo1* conditional deletion in the amygdala from birth impairs both LTP and basal synaptic transmission (9), and transsynaptic complexes at other hippocampal synapses also contribute to basal synaptic plasticity (4, 5). The molecular basis underlying the selective role of Nrxn-Cbln4-Neo1 in EC-GC LTP without affecting basal transmission is unknown. Assuming *Cbln4* and *Neo1* deletions do not affect other properties, *Cbln4* and *Neo1* cKO mice, by selectively blocking EC-GC LTP, may offer a unique opportunity to test the role of this form of plasticity in learning and memory.

The mechanism downstream of Neo1 that enables LTP at EC-GC synapses remains to be determined. Cbln4

binding to Neo1 could regulate the function and nanoscale localization of numerous molecules that control postsynaptic AMPAR levels and are essential to the core mechanisms of EC-GC LTP. Further work is required to identify this signaling cascade. Transsynaptic complexes may allow bidirectional signaling, and, therefore, the Nrxn-Cbln4-Neo1 complex at EC-GC synapses could also signal in a retrograde manner and contribute to potential structural (11) and functional (12) presynaptic changes associated with a postsynaptic form of plasticity, such as EC-GC LTP. Regardless of the precise signaling mechanism, the findings by Liakath-Ali et al. (1) not only support the synapse specificity of transsynaptic complexes but also highlight the functional relevance and diversity of this form of signaling at the synapse. Lastly, the findings also emphasize how molecules involved in synapse formation or partner specificity during development can be repurposed for synaptic plasticity in the mature brain.

As is often the case with intriguing results, these trigger more questions. Is the signaling mediated by transsynaptic adhesion complexes a general requirement for postsynaptic forms of LTP and LTD? Are these complexes also important for plasticity at other synapses not involving principal cells (e.g., inhibitory interneurons)? Are Cbln4 and other Cblns secreted in an activity-dependent manner? As mentioned above, how exactly do these transsynaptic adhesion complexes enable plasticity? While classical LTP seems to rely on a common core mechanism, a diverse transsynaptic regulatory plasticity mechanism may provide a way to manipulate synaptic plasticity, neuronal circuits, and behaviors in a synapse type-specific manner (13).

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1. K. Liakath-Ali, J. S. Polepalli, S.-J. Lee, J.-F. Cloutier, T. C. Südhof, Transsynaptic cerebellin 4-neogenin 1 signaling mediates LTP in the mouse dentate gyrus. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2123421119 (2022).
2. T. V. Bliss, T. Lomo, Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J. Physiol.* **232**, 331-356 (1973).
3. M. Jiang et al., Conditional ablation of neuroligin-1 in CA1 pyramidal neurons blocks LTP by a cell-autonomous NMDA receptor-independent mechanism. *Mol. Psychiatry* **22**, 375-383 (2017).
4. J. Dai, C. Patzke, K. Liakath-Ali, E. Seigneur, T. C. Südhof, GluD1 is a signal transduction device disguised as an ionotropic receptor. *Nature* **595**, 261-265 (2021).
5. W. Tao, J. Diaz-Alonso, N. Sheng, R. A. Nicoll, Postsynaptic $\delta 1$ glutamate receptor assembles and maintains hippocampal synapses via Cbln2 and neurexin. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E5373-E5381 (2018).
6. M. Yuzaki, A. R. Aricescu, A GluD coming-of-age story. *Trends Neurosci.* **40**, 138-150 (2017).
7. N. A. O'Rourke, N. C. Weiler, K. D. Micheva, S. J. Smith, Deep molecular diversity of mammalian synapses: Why it matters and how to measure it. *Nat. Rev. Neurosci.* **13**, 365-379 (2012).
8. D. Sun et al., Neogenin, a regulator of adult hippocampal neurogenesis, prevents depressive-like behavior. *Cell Death Dis.* **9**, 8 (2018).
9. X. D. Sun et al., Neogenin in amygdala for neuronal activity and information processing. *J. Neurosci.* **38**, 9600-9613 (2018).
10. X. Wu et al., Neuroligin-1 signaling controls LTP and NMDA receptors by distinct molecular pathways. *Neuron* **102**, 621-635.e3 (2019).
11. J. N. Bourne, K. M. Harris, Nanoscale analysis of structural synaptic plasticity. *Curr. Opin. Neurobiol.* **22**, 372-382 (2012).
12. M. Y. Min, F. Asztely, M. Kokaia, D. M. Kullmann, Long-term potentiation and dual-component quantal signaling in the dentate gyrus. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 4702-4707 (1998).
13. K. Suzuki et al., A synthetic synaptic organizer protein restores glutamatergic neuronal circuits. *Science* **369**, eabb4853 (2020).