Endocannabinoid Mediates Excitatory Synaptic Function of \( \beta \)-Neurexins. Commentary: \( \beta \)-Neurexins Control Neural Circuits by Regulating Synaptic Endocannabinoid Signaling

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Keywords: \( \beta \)-neurexins, endocannabinoid, synaptic plasticity, neural circuits, LTP, autism, Alzheimer disease, fragile X syndrome

A commentary on

\( \beta \)-Neurexins Control Neural Circuits by Regulating Synaptic Endocannabinoid Signaling
by Anderson, G. R., Aoto, J., Tabuchi, K., Foldy, C., Covy, J., Yee, A. X., et al. (2015). Cell 162, 593–606. doi: 10.1016/j.cell.2015.06.056

INTRODUCTION

Synaptic cell-adhesion molecules and their interactions with other molecular pathways affect both synapse formation and its function (Varoqueaux et al., 2006; Sudhof, 2008; Bemben et al., 2015a). Neurexins are presynaptic cell-adhesion molecules that interact with neuroligins and other postsynaptic partners. Neurexins are encoded by three genes, each of which encodes a long and short isoform, termed \( \alpha \)- and \( \beta \)-neurexins, respectively (Sudhof, 2008). Interestingly, despite studies linking neurexins to autism and other neuropsychiatric disorders (Leone et al., 2010; Rabaneda et al., 2014), the precise cellular mechanisms underlying the role of neurexins in cognition remain poorly understood.

Since most biochemical studies of neurexins have focused on \( \beta \)-neurexins, investigating the synaptic actions of \( \beta \)-neurexins is particularly imperative. In their timely Cell article, Anderson et al. reported that \( \beta \)-neurexins selectively modulate synaptic strength at excitatory synapses by regulating postsynaptic endocannabinoid synthesis, describing an unexpected trans-synaptic mechanism for \( \beta \)-neurexins to control neural circuits via endocannabinoid signaling (Anderson et al., 2015; Summarized in Figure 1A).

\( \beta \)-NEUREXINS REGULATE EXCITATORY NEUROTRANSMISSION VIA ENDOCANNABINOID SIGNALING

Functional study of neurexins represents a major technical challenge due to their diversity and complexity. To study the specific role of \( \beta \)-neurexins, Anderson et al. generated conditional knockout mice of all \( \beta \)-neurexin genes. Using electrophysiological and pharmacological
approaches, the authors elegantly analyzed neurotransmission and synaptic strength in preparations of cultured cortical neurons and acute subiculum slices from those β-neurexin knockout mice (Anderson et al., 2015).

Can β-neurexins be specifically involved in excitatory or inhibitory neurotransmission? In cultured cortical neurons, Anderson et al. found that β-neurexin knockout decreased the excitatory synapse parameters including AMPA receptor- and NMDA receptor-mediated excitatory postsynaptic currents (EPSCs), release probability and action-potential induced calcium influx, but had no effect on GABA receptor-mediated inhibitory postsynaptic currents (IPSCs; Anderson et al., 2015). Consistently, β-neurexin knockout decreased spontaneous miniature EPSCs (mEPSCs) and lowered the surface GluA1 AMPARs, but had no effect on miniature IPSCs (mIPSCs) (Anderson et al., 2015). These data indicate that β-neurexins are selectively essential for neurotransmission at excitatory synapses. Importantly, the impaired mEPSCs could be rescued by re-expression of neurexin-1β, but not by increased expression of neurexin-1α, suggesting that modulation of excitatory neurotransmission by β-neurexins, despite their lower abundance, is independent of α-neurexins (Anderson et al., 2015).

How can β-neurexins modulate excitatory transmission? As their previous study has suggested that neurexin-3 is specifically required for tonic endocannabinoid signaling at inhibitory synapses (Foldy et al., 2013), Anderson et al. hypothesized that β-neurexins, the presynaptic interactor of neurexin-3, might regulate neurotransmission via endocannabinoid system. To test this, the authors pharmacologically manipulated the endocannabinoid system in cultured cortical neurons. Indeed, treatment with a cannabinoid receptor 1 (CB1R) antagonist, enhanced the mEPSC frequency in β-neurexin knockout neurons, but had no effect in control neurons; the CB1R agonist caused less decrease in mEPSC frequency in β-neurexin knockout neurons than in control ones (Anderson et al., 2015). These findings indicate that β-neurexin knockout enhances basal endocannabinoid tone and
tonic presynaptic CB1R activation, further revealing a link of the neurexins/neuroligins complex to endocannabinoid signaling. As presynaptic CB1R activation are known to inhibit presynaptic Ca$^{2+}$ channels and decrease neurotransmitter release (Castillo et al., 2012), the authors conclude that β-neurexins might control excitatory neurotransmission through downregulating endocannabinoid system and the impaired excitatory neurotransmitter release is at least partially due to enhanced endocannabinoid signaling in absence of β-neurexins (Anderson et al., 2015).

How does β-neurexin knockout increase tonic endocannabinoid signaling at excitatory synapses? The examination of CB1R levels detected no changes in β-neurexin knockout neurons (Anderson et al., 2015), suggesting that β-neurexin knockout may affect endocannabinoid synthesis. To identify which of the two major endocannabinoids—2-arachidonoylglycerol (2-AG) and anandamide—is affected by β-neurexin knockout, Anderson et al. compared the effects of bath application of each endocannabinoid, and found that the enhanced endocannabinoid tone might be caused by the increase of 2-AG as exogenous 2-AG produced little additional inhibition on mEPSCs in β-neurexin knockout neurons. 2-AG is synthesized via a postsynaptic phospholipase C-dependent pathway (Anderson et al., 2015). Unsurprisingly, inhibition of 2-AG synthesis in postsynaptic neurons with phospholipase C inhibitor rescued mEPSC frequency and restored the sensitivity of CB1Rs to exogenous 2-AG in β-neurexin knockout neurons, further confirming that loss of β-neurexins cause synaptic phenotypes via presynaptic CB1R activation by elevated postsynaptic 2-AG production (Anderson et al., 2015). Notably, the postsynaptic partners of β-neurexins in regulating endocannabinoid synthesis remain unknown (Figure 1A).

Impressively, in acute subiculum slices, Anderson et al. found that presynaptic β-neurexin knockout in CA1 pyramidal neurons selectively decreases excitatory synaptic strength at burst-firing subiculum neurons, at least in part, by enhancing tonic endocannabinoid signaling, indicating that β-neurexins also control endocannabinoid system in vivo (Anderson et al., 2015). Particularly, β-neurexin knockout selectively blocked long-term potentiation (LTP) in burst-firing neurons (Anderson et al., 2015). LTP is induced by presynaptic activation of PKA in burst-firing neurons of the subiculum (Wozny et al., 2008). Activation of CB1Rs, which are Gi/o protein-coupled receptors, inhibits adenyl cyclases/PKA (Castillo et al., 2012) and possibly blocks presynaptic LTP. The authors next demonstrated both CB1R antagonist and 2-AG synthesis inhibitor rescued the LTP impairment caused by β-neurexin knockout, firstly linking endocannabinoid signaling to presynaptic LTP of excitatory synapses (Anderson et al., 2015; Figure 1A). Further research is needed to investigate the mechanism underlying the cell-specific function of β-neurexins in burst-firing neurons relative to regular-firing ones.

Finally, the authors showed that deleting β-neurexins from the hippocampal CA1 region selectively impaired mouse contextual fear memory, indicating that β-neurexins in hippocampal CA1 neurons is important for learning and memory (Anderson et al., 2015). However, the behavioral evidence is still limited. Additionally, the authors did not confirm the involvement of endocannabinoid system in behavioral deficits caused by hippocampal β-neurexin knockout.

Altogether, Anderson et al. exquisitely revealed that β-neurexins have a unique role in transsynaptic modulation of endocannabinoid tone at excitatory synapses, which is essential for synaptic plasticity and behaviors, thus mechanistically linking β-neurexins to cognitive function (Anderson et al., 2015).

**FUTURE PERSPECTIVE**

Investigating the synaptic function of neurexins/neuroligins is crucial to elucidate the pathomechanisms of diseases associated with these cell-adhesion molecules. The discovery of transsynaptic modulation of endocannabinoid signaling by β-neurexins, not only provides insights into the molecular mechanisms underlying neural circuits, but also helps understand synaptopathies in cognitive diseases.

Endocannabinoid system regulates neural circuits and offers therapeutic opportunities for neuropsychiatric diseases (Castillo et al., 2012; Wyrofsky et al., 2015). The neurexins/neuroligins-endocannabinoid signaling pathway likely modulates circuit dynamics in distinct brain regions and may implicate many brain disorders (Figure 1B). The conditional knockout mice combined with other genetic or pharmacological approaches will provide useful tools for investigating this pathway in neural circuits and its behavioral and therapeutic relevance. Much more work will be required, but the study highlighted herein is encouraging in this direction.

**AUTHOR CONTRIBUTIONS**

The author confirms being the sole contributor of this work and approved it for publication.

**ACKNOWLEDGMENTS**

HW was supported by the National Natural Science Foundation of China (NSFC, No.30200152) for Rett syndrome studies and the Fragile X Research Foundation of Canada.

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Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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