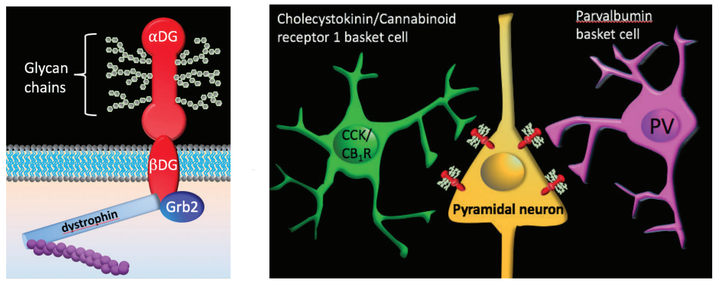
Neuronal dystroglycan regulates CCK/CB1R interneuron development

Daniel S. Miller[1] and Kevin Wright[2]

[1]Neuroscience Graduate Program, [2]Vollum Institute, Oregon Health and Sciences University

Abstract

Dystroglycan is a transmembrane glycoprotein receptor that links the extracellular matrix to the actin cytoskeleton through its two covalently associated subunits, a heavily glycosylated extracellular subunit alpha dystroglycan (a-DG), and the membrane spanning beta-dystroglycan (b-DG). Glycosylation of dystroglycan is essential for its ability to bind extracellular matrix ligands. Mutations in multiple glycosyltransferases lead to hypoglycosylated dystroglycan and underlie a family of congenital muscular dystrophies characterized by muscle weakness, brain malformations, and neurological symptoms such as seizures. The role of dystroglycan in maintaining the radial glial scaffold during early cortical development and its contribution to disease pathology is well studied, whereas the function of dystroglycan in postmitotic neurons has remained elusive. Dystroglycan is expressed throughout adulthood on excitatory pyramidal neurons and is closely associated with inhibitory synapses, suggesting a role for dystroglycan in synapse function or development. Recently, dystroglycan was found to regulate the formation of CCK/CB1R+ interneurons, one of two major basket cell populations that provide inhibitory input to pyramidal cells of the cortex and hippocampus. However, the mechanisms by which dystroglycan orchestrates development and formation of this specific inhibitory synapse remain essentially unknown. Using a novel hypomorphic mouse model of dystroglycanopathy (*B3gnt1*), conditional deletion of dystroglycan (Nex^{Cre}; $DG^{F/-}$) or its intracellular signaling domain ($DG^{F/\Delta}$; Nex^{Cre}), we show that CCK/CB1R interneurons disappear between the first and second postnatal week after birth. Furthermore, maintenance of CCK/CB1R+ terminals does not require full glycosylation of a-DG or the intracellular signaling domain of b-DG. These results point to a critical role for dystroglycan function in neurons during inhibitory synapse development.



Selective loss of CB1R interneuron terminals in mice lacking neuronal dystroglycan

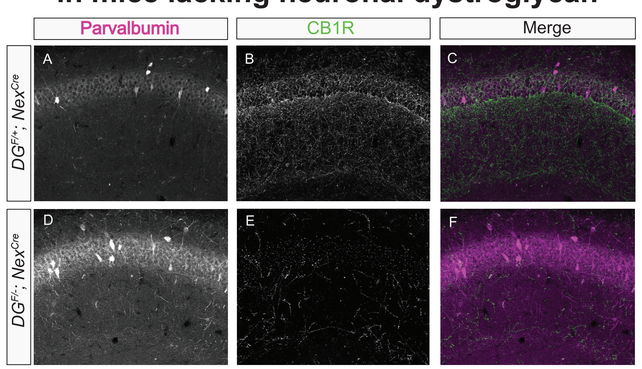


Fig 1: Immunohistochemical staining of parvalbumin **(A, D)** and cannabinoid receptor 1 expressing inhibitory interneuron terminals **(B, E)** in hippocampus of adult control **(A-C)** and dystroglycan conditional knockout mice **(D-F)**. Although both interneuron subtypes provide perisomatic innervation to excitatory pyramidal neurons, mice lacking dystroglycan in pyramidal neurons ($DG^{F/-}$; Nex^{Cre}) show a selective disappearance of CB1R+ interneuron terminals **(E)** compared with PV+ interneurons **(D)**.

Developmental timecourse of CCK/CB1R+ presynaptic terminal loss

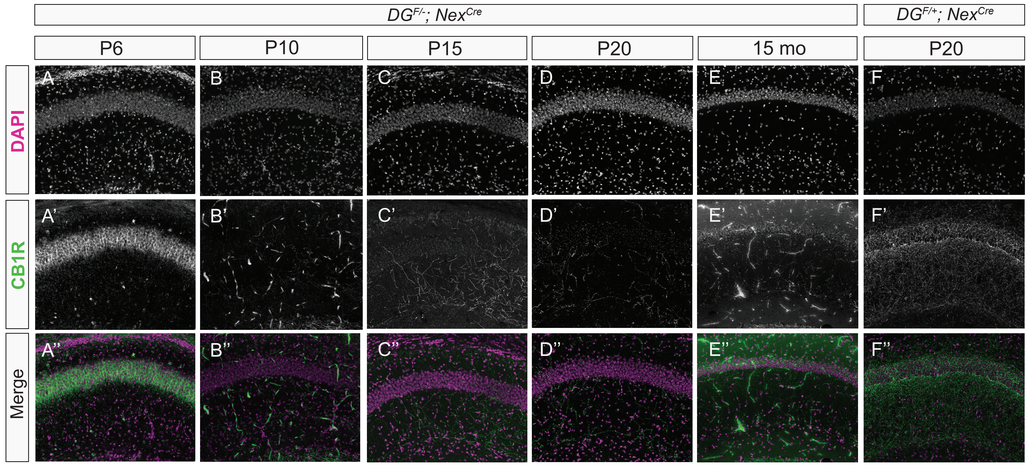


Fig 2: Immunohistochemical detection of cannabinoid receptor expressing (green) interneuron terminals and nuclei (DAPI, magenta) in the CA1 of mice lacking dystroglycan in pyramidal neurons ($DG^{F/-}$; Nex^{Cre}) during postnatal development **(A-E)**. Compared with control mice at postnatal day 20 (P20) **(F-F")**, CB1R expression in DG cKO mice decreases to nearly undetectable levels between P6 **(A-A")** and P10 **(B-B")**. This reduction in CB1R expression persists at later developmental ages up to 15 mo **(C"-E")**.

CCK/CB1R+ terminals are retained in hypoglycosylation mutant mice

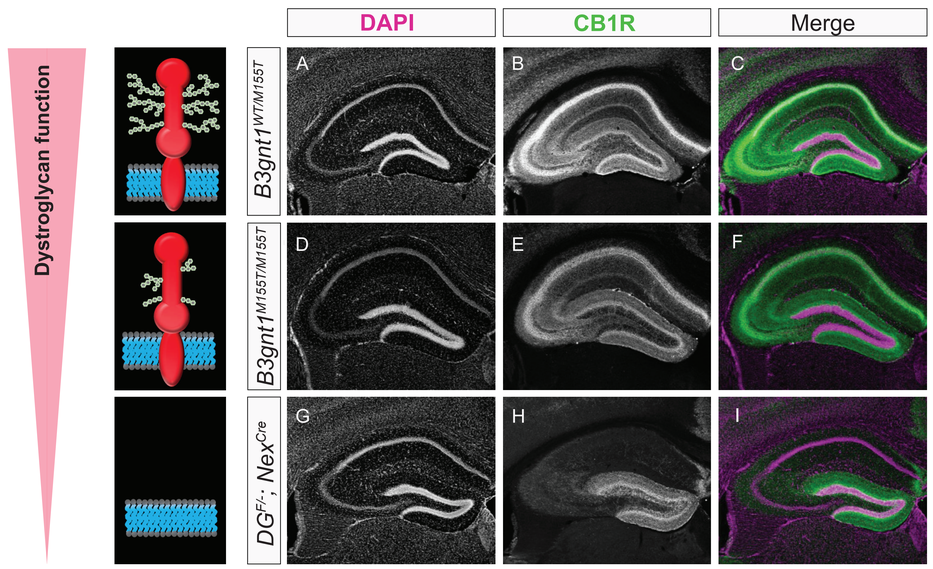


Fig 3: Adult mice lacking functional dystroglycan in pyramidal neurons ($DG^{F/-}$; Nex^{Cre}, bottom row) have fewer CB1R+ terminals (green) in hippocampal CA regions **(H, I)**, compared with $B3gnt1^{WT/M155T}$ mice containing wild-type levels of functional, glycosylated dystroglycan **(B, C)**. Hypomorphic $B3gnt1^{M155T/M155T}$ mutant mice contain similar numbers of CB1R+ terminals to control animals **(E, F)** despite having partially reduced levels of glycosylated dystroglycan. The *NEX* promoter is highly expressed in CA regions of the hippocampus but spares the dentate gyrus, leaving CB1R expression unaltered in the dentate gyrus of DG cKO mice **(H, I)**.

Beta-dystroglycan is dispensable for CCK/CB1R+ interneuron development

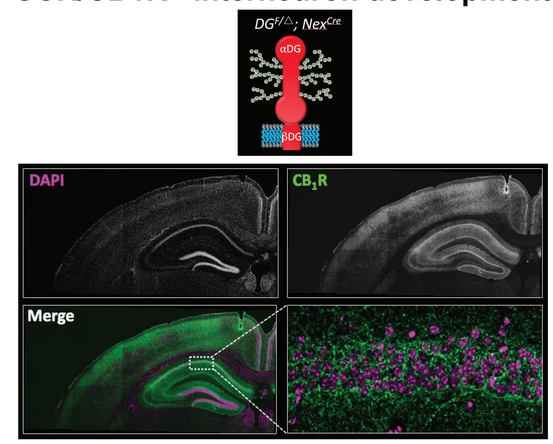


Fig 4: The cortex and hippocampus develop normal morphology in mice lacking the intracellular signaling domain of β-DG in pyramidal neurons ($DG^{F/\Delta}$; Nex^{Cre}). Immunohistochemical staining of cannabinoid receptor 1 expression (green) shows a normal pattern, with abundant CB1R terminals forming perisomatic synapses (boxed image, CA1).

Conclusions and Future Directions

Conclusions:

- Deletion of neuronal dystroglycan leads to a specific loss of CB1R+ perisomatic innervation of pyramidal neurons, whereas innervation by PV+ interneurons remains intact
- Partial reduction of glycosylated dystroglycan is not sufficient to disrupt CB1R+ interneuron synapse formation
- The intracellular signaling domain of β-DG is not required for CCK/CB1R+ synapses to form during development

Future directions:

- Determine whether apparent loss of CCK/CB1R+ terminals in DG cKO mice is due to cell death, retraction of synaptic contacts, or down-regulation of gene expression
- Assess how loss of dystroglycan function affects seizure susceptibility in DG cKO mice and a clinically relevant mouse model of dystroglycanopathy (*B3gnt1*)
- Identify candidate presynaptic ligands for dystroglycan at CCK/CB1R+ interneuron synapses

Acknowledgments

I would like to thank my mentor Kevin Wright and the members of the Wright lab for their assistance and feedback. This work was funded by an R01 (NS091027) to K.W. and an ARCS Foundation Scholarship awarded to D.M.