Investigating the vesicular transport mechanisms of myelin proteolipid protein 1 (PLP1) in optic nerve myelination

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INTRODUCTION

Myelination allows rapid axonal information transduction. Proteolipid protein (PLP) insertion into myelin is important to maintain compact myelin structure and axonal integrity. However, the critical SNAREs involved in PLP vesicular transport remain unknown.

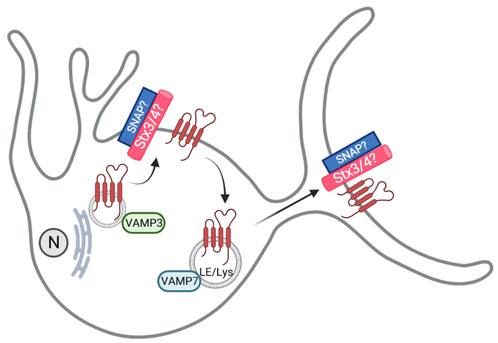

Figure 1. PLP trafficking in oligodendrocytes. *In vitro* studies suggest syntaxin-3 as the key target SNARE for PLP transport to the cell membrane [1].

Hypothesis: Based on in vitro studies and syntaxin-3 mRNA localization in the myelin [2], we hypothesized that Syntaxin-3 acts as the t-SNARE to transport PLP to the cell and myelin membrane.

METHODS

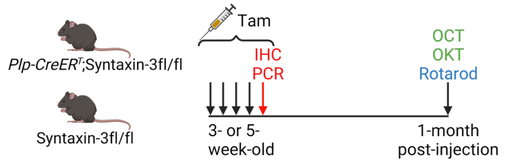

Figure 2. Animal models and study design for Syntaxin-3 removal in oligodendrocytes. *Plp-CreER^T* expresses tammoxifen-inducible Cre in oligodendrocytes. Tamoxifen was injected for 5 consecutive days at 3-week-old (1mg) or 5-week-old (2mg) Cre expressing mice (Stx3 cKO) and Syntaxin-3 floxed mice as control.

RESULTS

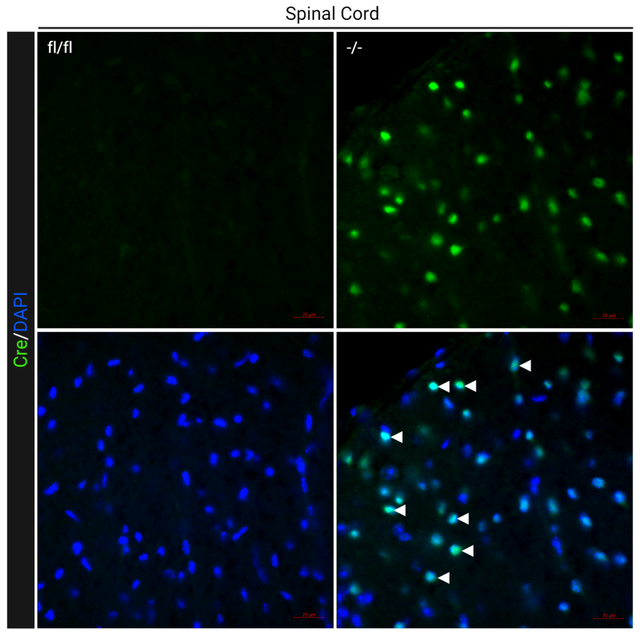

Figure 3. Cre translocation to the nucleus with tamoxifen injection. Confocal images of the spinal cord white matter showing Cre (labeled with GFP) and DAPI staining. Control (fl/fl) and Stx3cKO (-/-) tissues were obtained immediately after day 5 tamoxifen injection. White arrows show examples of colocalization of Cre and DAPI. Scale bar, 20μm.

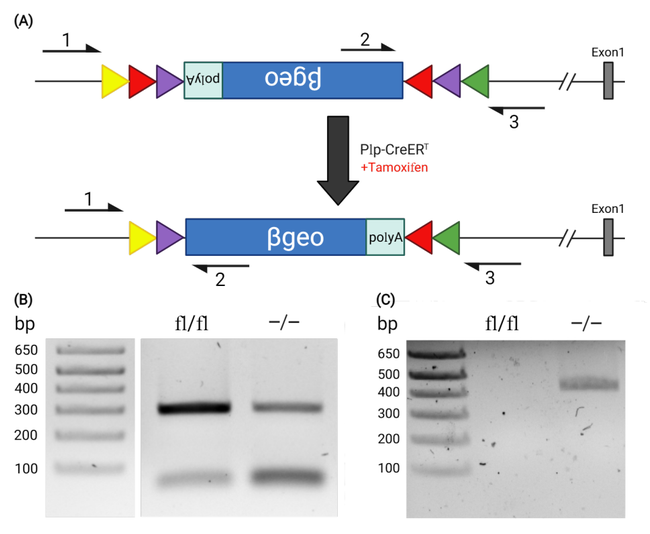

Figure 4. Stx3 cKO generation. (A) Cre-FLEX system of inducing Stx3 cKO. Inverted β-galactosidase (βgeo) and polyA tail are flanked by 2 inverted lox sequences, loxp (purple) and lox511 (red). Upon tamoxifen administration, Cre inverts the flanked sequence to disrupt downstream exons transcription. Arrows: PCR primers design. Yellow, FRT; Green, F3. **(B)** PCR product of the floxed allele (primers 2 and 3). **(C)** PCR product of the knockout allele (primers 1 and 2). Tissues were obtained from the brain and spinal cord.

RESULTS

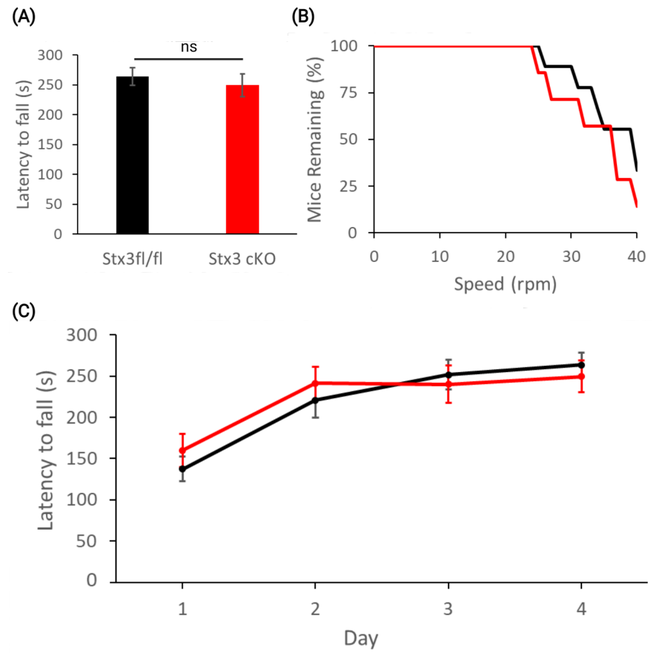

Figure 5. Rotarod Test. (A) No significant difference in latency to fall between groups (Student *t*-test). **(B)** Speed (rotation per minute, rpm) at which mice fell **(C)** Rotarod experiment learning curve over the 4-day period. Error bar: SEM. Red: Stx3cKO, Black: Stx3fl/fl

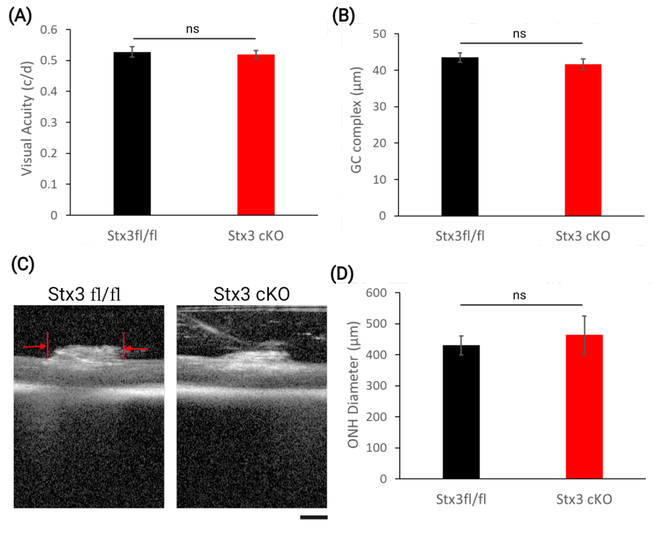

Figure 6. No visual impairment in Stx3 cKO. (A) The o ptokinetic test (OKT) shows no significant difference between the tro groups in visual acuity. **(B)** Optic coherence tomography (OCT) (n=3 for each group). The thickness of the ganglion cell (GC) complex is not significantly different between control and Stx3 cKO. **(C)** OCT images show an example of measuring ONH size. Scale bar, 200μm. **(D)** Optic nerve head sizes does not differ between control and Syntaxin-3 cKO animals. Bars show mean±SEM.

CONCLUSION

Syntaxin-3 cKO in oligodendrocytes in adult mice does not impair central nervous system myelination.

However, adult myelin is relatively stable, and the half-life of PLP in myelin is approximately 6 months [3]. Longitudinal tracking will be necessary to evaluate the role of Syntaxin-3 in myelin maintenance.

Clinical relevance: PLP involves in hypomyelinating disease Pelizaeus-Merzbacher Disease (PMD) and axonal degenerating disease Spastic paraplegia 2 (SPG2) [4]. Identifying the trafficking pathway of PLP can advance novel treatment strategies in myelin regeneration

FUTURE DIRECTIONS

Optic nerve myelination: Electrophysiology and electron microscopy.

PLP trafficking: IHC on PLP in myelin structures and quantification by Western blot.

Other SNAREs: Involvement of Syntaxin-4? SNAP-25 family proteins?

Myelin formation: Utilize another Cre line, *Olig2-Cre*, which induces KO in oligodendrocytes during development to study PLP trafficking in myelin development.

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