Investigating the expression of $GABA_A$ receptor subunits in $CDKL5^{-/y}$ rats to understand seizure mechanisms in CDKL5 Deficiency Disorder (CDD)

Maisie Taylor[1], Mohammad Sarfaraz Nawaz [1,2], Emma Perkins[1,2], Peter Kind[1,2]

1-INCR, University of Edinburgh, Edinburgh

2-Simons Initiative for the Developing Brain, Patrick Wild Centre, University of Edinburgh

Institute for Neuroscience and Cardiovascular Research

Introduction

CDD is a severe neurodevelopmental disorder in humans, typically characterised by early-onset epileptic seizures and intellectual disability (Zhu and Xiong, 2019). The loss of CDKL5 during development is believed to prevent the correct formation of GABAergic networks across various brain regions and contribute to neuronal hyperexcitability in pyramidal neurons of the piriform cortex (Sivilia et al., 2016). However, $CDKL5^{-/y}$ rats exhibit resistance to seizures, suggesting the involvement of compensatory inhibitory mechanisms different from humans. Unpublished data has shown that thalamic neurons from $CDKL5^{-/y}$ rats display increased tonic GABAergic currents (Figure 1). This increase could reflect either a greater number of $GABA_A$ receptors or enhanced receptor activity.

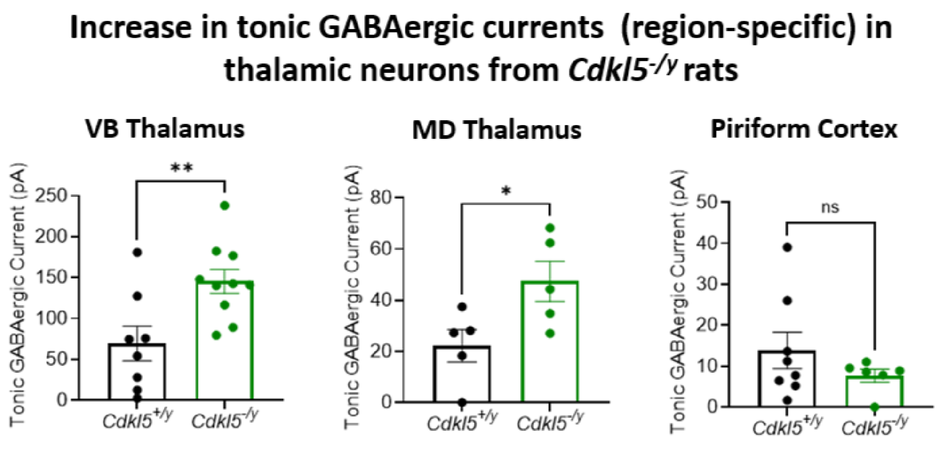


Figure 1- Unpublished data showing significantly higher GABAergic currents in $CDKL5^{-/y}$ VB thalamus and MD thalamus compared to their $CDKL5^{+/y}$ controls

Tonic currents are primarily mediated by extrasynaptic $GABA_A$ receptors, whose subunit composition determines their localisation and function (Table 1). Therefore, this project aims to investigate the role of these subunits in shaping GABAergic currents and inhibitory signalling, and to determine how their expression differs in $CDKL5^{-/y}$ rats compared to littermate controls, potentially explaining their seizure resistance.

$GABA_A$ Receptor Subunit	Localisation
α1	Synaptic
γ2	Synaptic & Extrasynaptic
δ	Extrasynaptic

Table 1- Localisation of $GABA_A$ receptor subunits associated with synaptic and extrasynaptic inhibition.

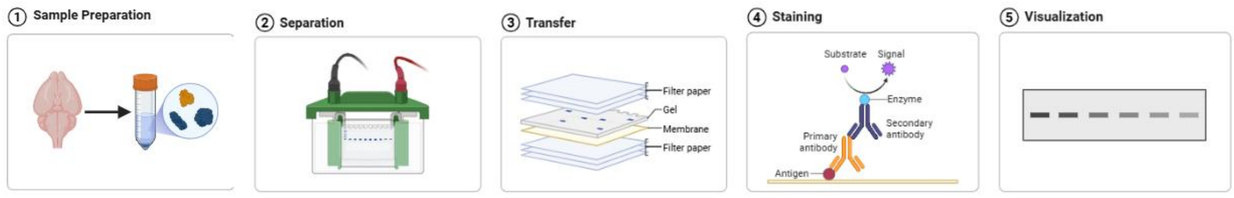
Aims

1) Use Western blot analysis and immunohistochemistry to quantify the levels of $GABA_A$ receptor subunits (α1, γ2, and δ) in the piriform cortex, hippocampus, thalamus, and cortex of *CDKL5*$^{-/y}$ rats and littermate controls.

2) Determine whether α1, γ2, and δ $GABA_A$ receptor subunits contribute to GABAergic inhibition in *CDKL5*$^{-/y}$ rats, and whether this controls the observed seizure resistance.

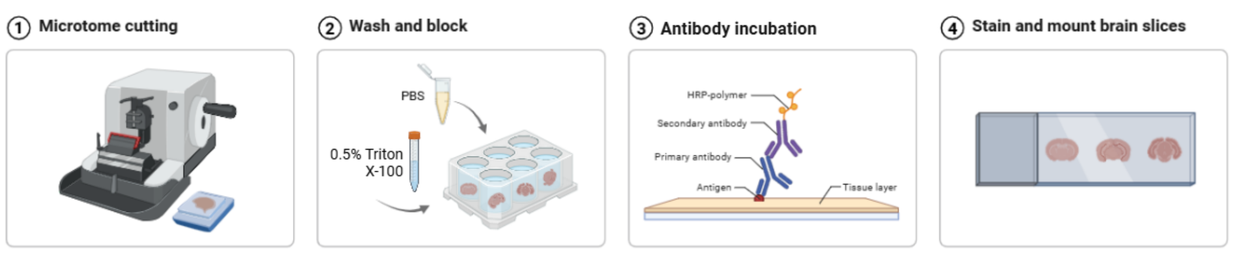
Methods

Western blot analysis



Protein samples were prepared and loaded into SDS-PAGE gels for electrophoresis. Following gel separation, proteins were transferred to a membrane, blocked to prevent non-specific binding, and incubated with α1, γ2, and δ antibodies. Membranes were then incubated with appropriate secondary antibodies and subsequently imaged using the LI-COR Odyssey system.

Immunohistochemistry



Methods (continued)

Brains were perfused, post-fixed, and stored in buffer at 4˚C prior to sectioning. Free-floating sections were washed, blocked, and incubated with α1, γ2, and δ antibodies overnight at 4˚C. Following washes, sections were then incubated with the appropriate secondary antibodies at room temperature. Finally, tissue was mounted, coverslipped, and stored at 4°C until imaging.

Results

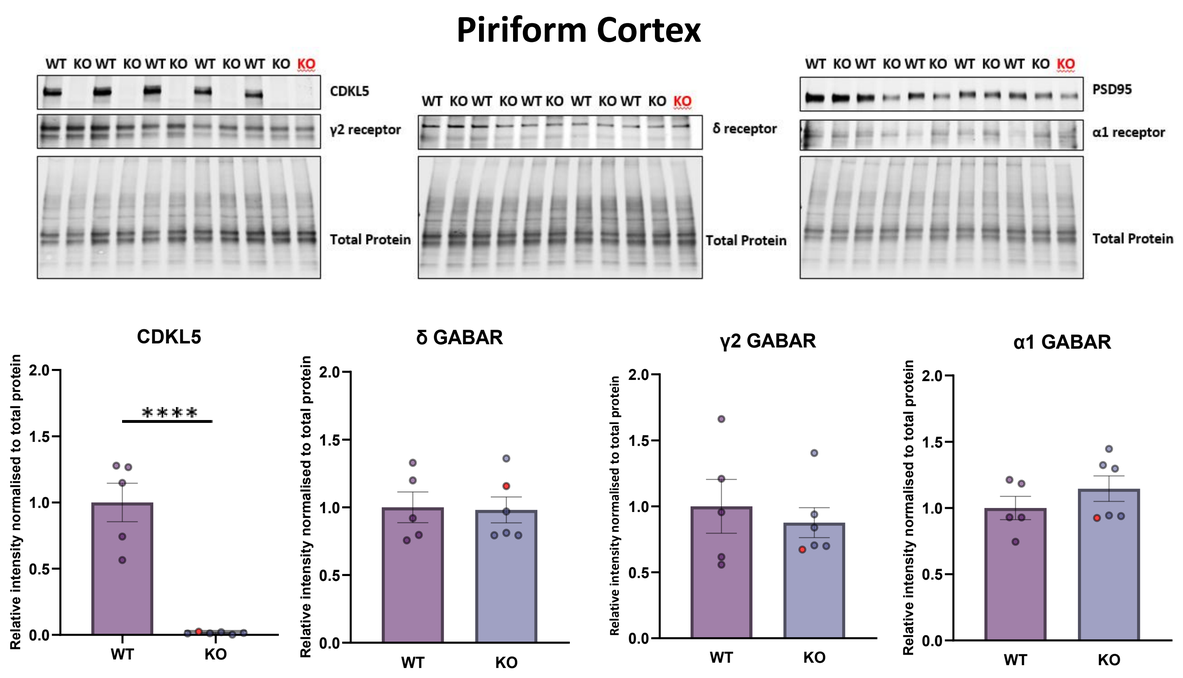


Figure 2- $CDKL5^{-/y}$ rats exhibit no change in the expression of $GABA_A$ receptor subunits in piriform cortex. Representative western blots showing expression level of γ2, δ, α1 subunits and PSD95 normalised to total protein. Cdkl5 protein is absent in $CDKL5^{-/y}$ rats. Spontaneous seizure $CDKL5^{-/y}$ rat shown in red.

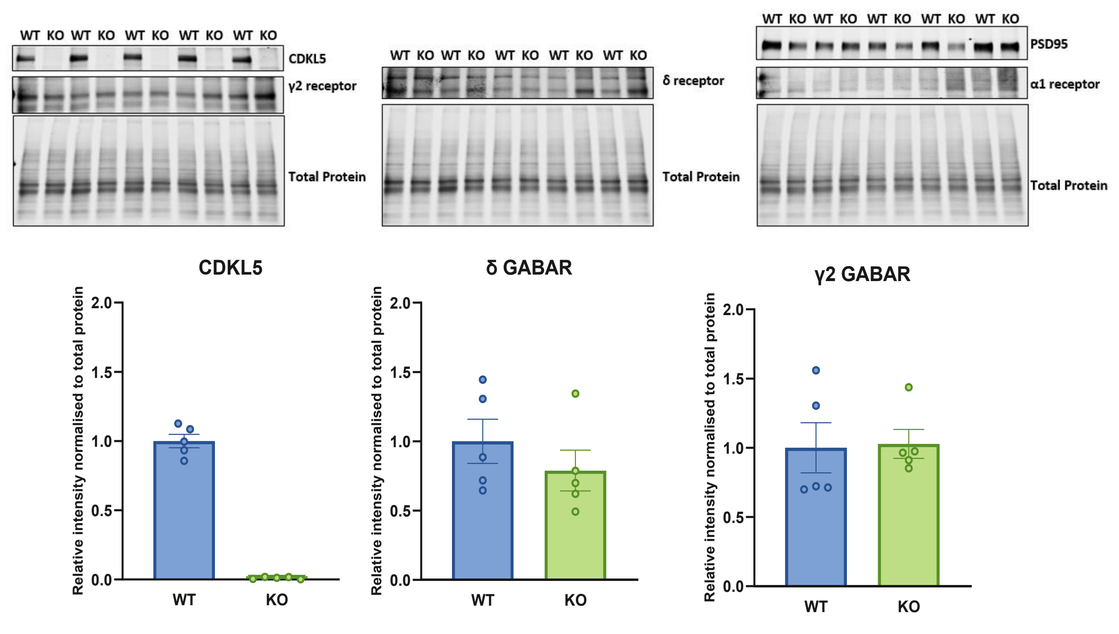


Figure 3- $CDKL5^{-/y}$ rats exhibit no change in the expression of $GABA_A$ receptor subunits in thalamus Representative western blots showing expression level of γ2, δ, α1 subunits and PSD95 normalised to total protein. Cdkl5 protein is absent in $CDKL5^{-/y}$ rats

Conclusions

1) Preliminary immunohistochemistry data has been collected, but the protocols still need optimisation to determine whether there's differences in the expression of α1, γ2, and δ $GABA_A$ receptor subunits.

2) Western blot analysis showed no significant differences in subunits examined between $CDKL5^{-/y}$ rats and controls across the brain regions, suggesting that seizure resistance is unlikely to be due to changes in these subunits and may involve other $GABA_A$ subunits or compensatory changes in inhibitory networks.

3) Further experiments examining broader ranges of $GABA_A$ subunits and functional measures of tonic inhibition are needed to further understand seizure resistance in rat models of CDD

References and Acknowledgments

Figures in methods section Created with BioRender.com

Sivilia, S. *et al.* (2016) 'CDKL5 knockout leads to altered inhibitory transmission in the cerebellum of adult mice', *Genes, Brain and Behavior*, 15(5), pp. 491–502. Available at: https://doi.org/10.1111/gbb.12292.

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Special thanks to Aneta Luczak-Stecura for microtome cutting training. Thank you to the BMTO for funding this summer vacation internship