Characterising the Synaptic Dysfunction of the Ubiquitin Proteasome System in Fragile X Syndrome

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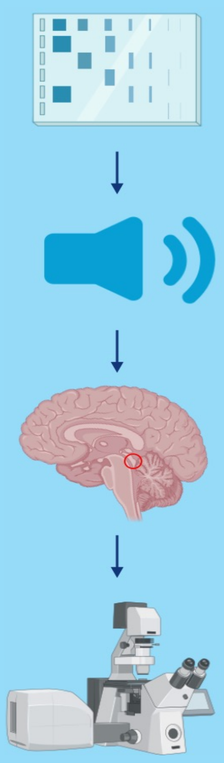
1. Introduction

- Fragile X Syndrome is caused by mutations in Fragile X Messenger Ribonucleoprotein 1 (*Fmr1* gene), and is the leading monogenic cause of autism spectrum disorder (Stone et al., 2023)
- The ubiquitin proteasome system (UPS) is involved in the hyperexcitability of *Fmr1*$^{-/y}$ mice after acoustic stimulation (used to model audiogenic seizures); a phenotype that is corrected by administering the proteasome inhibitor Bortezomib (BTZ) (Louros et al., 2023)
- My project assessed how UPS dysfunction contributes to the hyperexcitability in FXS, particularly in the inferior colliculus (IC). *Fmr1*$^{-/y}$ mice were crossed with lines expressing Ub^{G76V}-Green Fluorescent Protein (GFP), a reporter first described by Lindsten et al (2003)
- We aimed to identify the neurons where BTZ administration inhibited the activity of the proteasome, leading to the accumulation of Ub^{G76V}-GFP

2. Methodology



Genotyping – Confirm if the offspring are wildtype (WT) or mutant (*Fmr1*$^{-/y}$), and positive or negative for GFP expression using PCR and gel electrophoresis

Sound Stimulation – Subject the offspring to the Audiogenic Stimulation Test (115db for 30 seconds), one hour post injection with vehicle or BTZ

Staining – Collect 50µm sagittal sections using the freezing microtome and immuno-stain for NeuN and GFP, then mount onto microscope slides

Confocal Microscopy – Take Z-stacks of the IC then use FIJI software for qualitative analysis, colouring NeuN expression red and GFP expression green

Figure 1. Schematic of methodology used to assess UPS dysfunction. Created in BioRender [10/08/24]

3. Ub^{G76V}-GFP reporter is functional

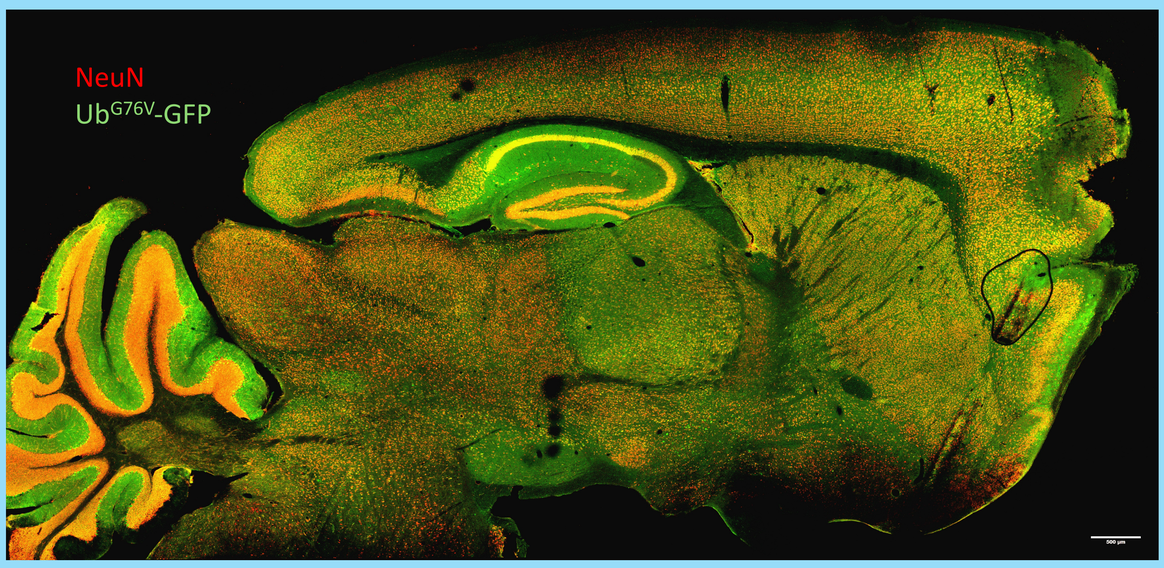


Figure 2. Neuronal expression of the proteasome reporter in the new FX-Ub^{G76V}-GFP mouse line

4. BTZ administration increases GFP expression in *Fmr1*$^{-/y}$ IC

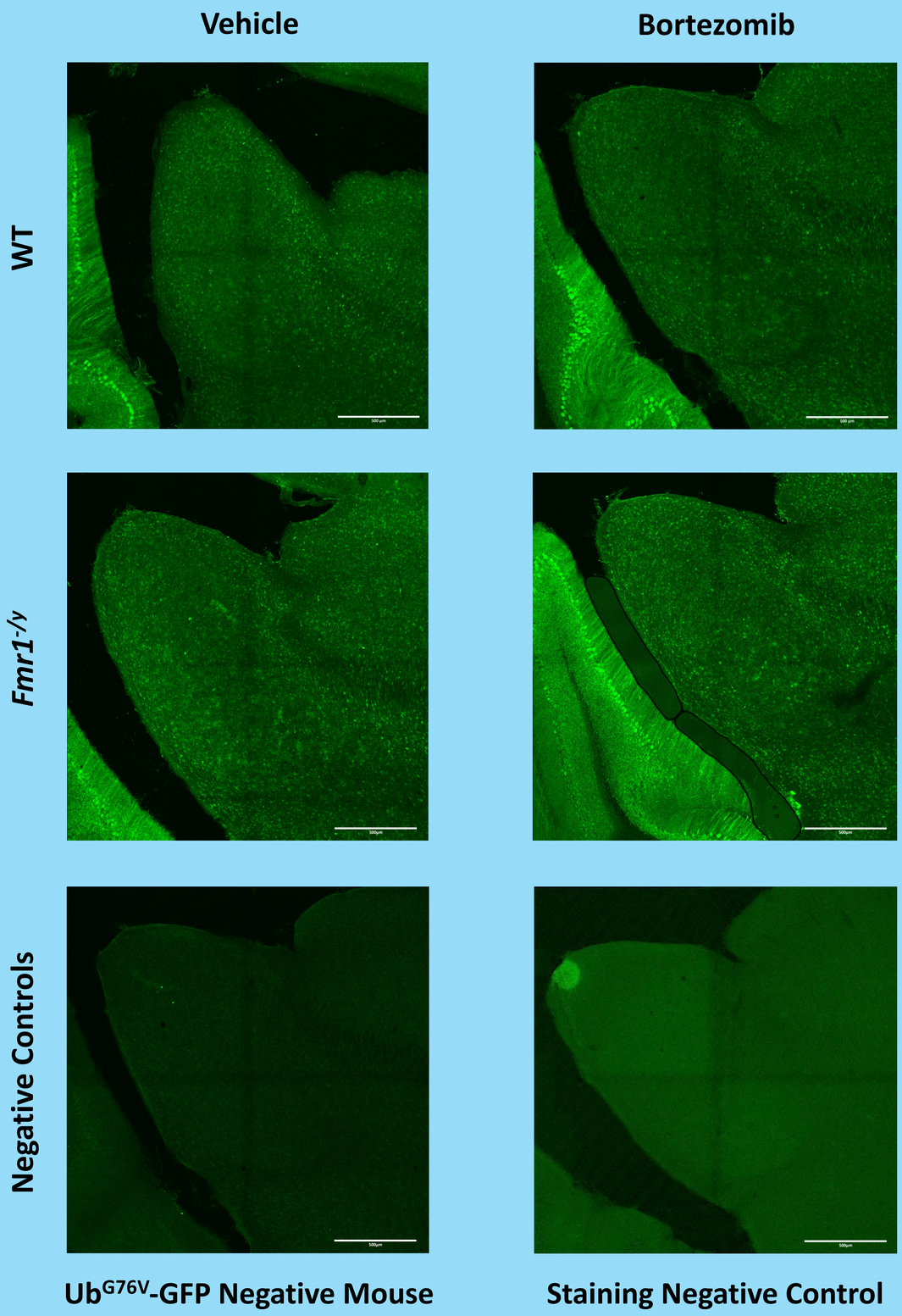


Figure 3. Ub^{G76V}-GFP expression in the inferior colliculus (IC) of wild-type (WT) and *Fmr1*$^{-/y}$ mice, following injection with vehicle or Bortezomib. Proteasome inhibition causes a visible increase in Ub^{G76V}-GFP expression in the *Fmr1*$^{-/y}$ mouse

5. Future Directions

- Fmr1$^{-/y}$ mice exhibit a higher expression of GFP, indicating proteasomal inhibition causes impaired GFP degradation
- Future experiments should use Imaris software to quantitatively analyse GFP expression, and determine whether the differences between WT and Fmr1$^{-/y}$ colonies are significant
- Tissue sections should be triple immunostained for NeuN, GFP, and cFos (neurone activation marker). Observing co-localisation between cFos and GFP expression would indicate that proteasomal activity is more inhibited in hyperactivated neurones, generating a novel target for the treatment of Fragile X Syndrome

6. References

- Lindsten, K., Menéndez-Benito, V., Masucci, M. G., & Dantuma, N. P. (2003). A transgenic mouse model of the ubiquitin/proteasome system. Nature biotechnology, 21(8), 897–902, https://doi.org/10.1038/nbt851
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