Fragile-x Syndrome Interneuron Density Alterations Post Early Life Pharmacological Intervention

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Background And Hypothesis:

Excessive neuronal excitability in Fragile-X syndrome (FXS) alters interneuron (IN) cell densities in the somatosensory cortex. This excitability and cell density alteration has been linked to lower cAMP levels (Berry-Kravis E. *et al.*, 1995) . Interventions to increase cAMP have improved cognition in FXS patients (Chadwick W., *et al.* 2024)

Therefore, we hypothesised that the phosphodiesterase inhibitor BPN14770 would recover IN cell densities to wild-type levels.

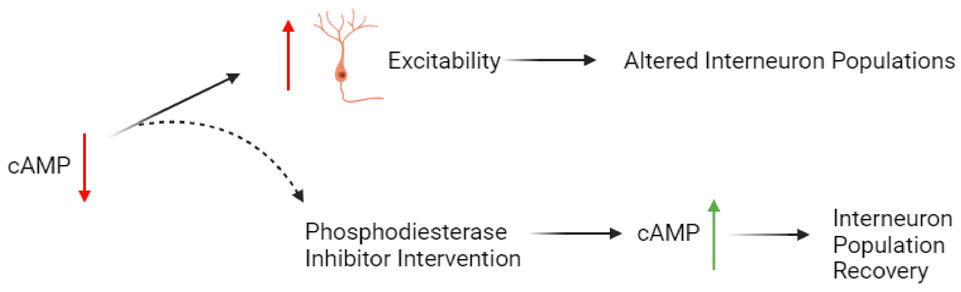

Figure 1. Theory of phosphodiesterase inhibitor BPN14770 action in Fragile-X syndrome rats. Created in Biorender.com.

Methods:

Cortical rat brain slices were sliced using a cryo-microtome. Cortical slices were then separated for different secondary antibody staining protocols, one for parvalbumin interneuron staining and another for somatostatin interneurons.

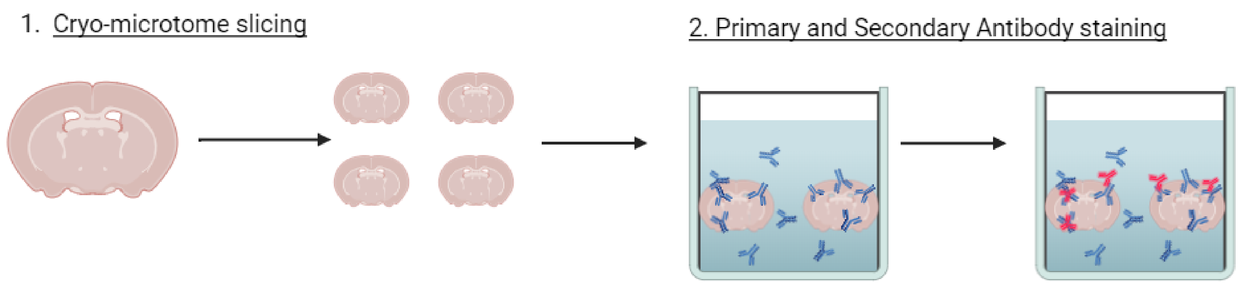

Figure 2. Cryo-microtome and secondary antibody staining protocol. Created in Biorender.com.

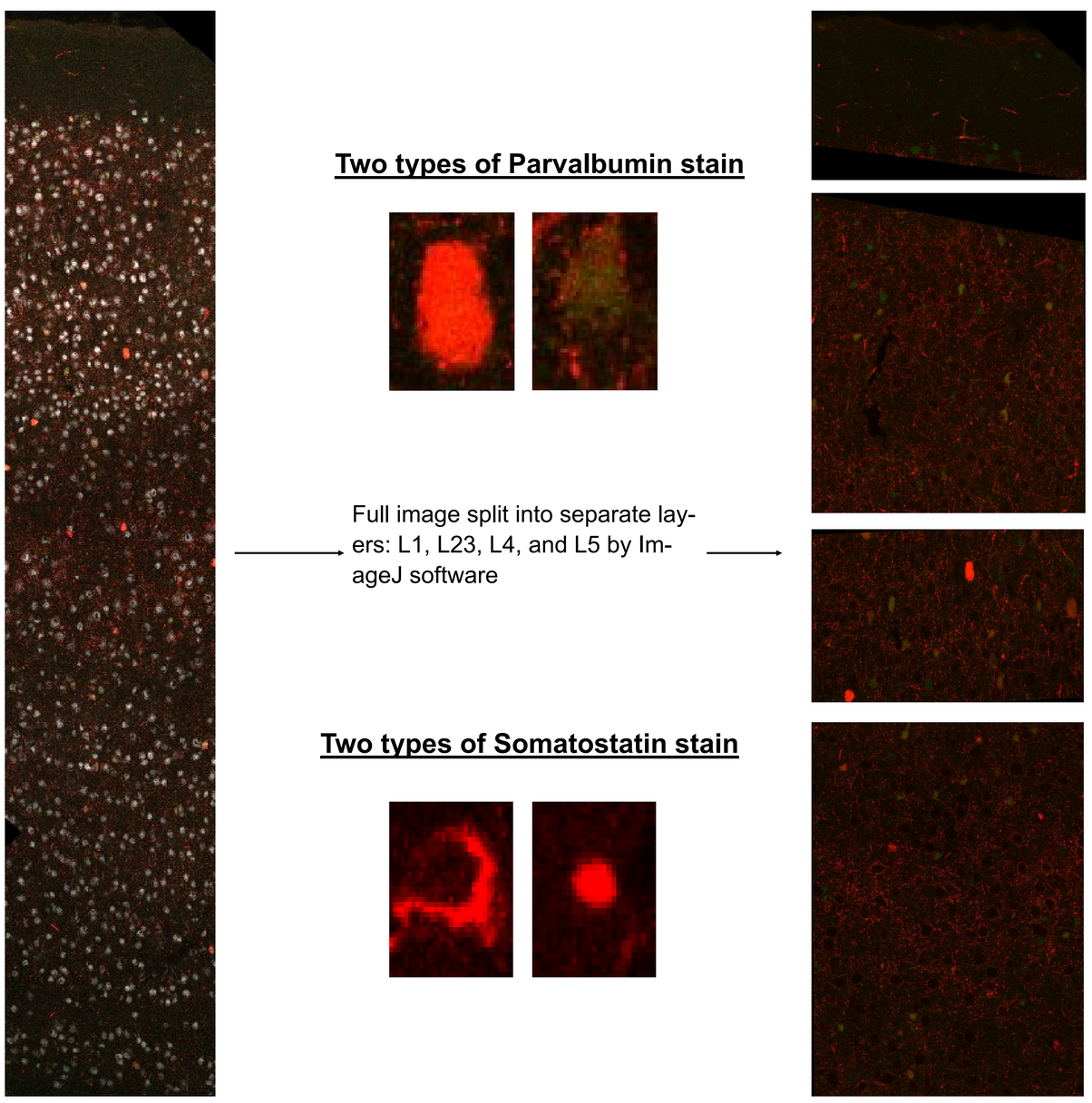

Figure 3. Processing of immuno-stained rat brain slice images using ImageJ, and exemplar parvalbumin and somatostatin cell staining and imaging.

Cortical slices were imaged for the S1 region of the somatosensory cortex. Images were used to calculate parvalbumin, somatostatin and total IN densities of each S1 layer using ImageJ software.

References:

Berry-Kravis E., Hicar M., Ciurlionis R. (1995). Reduced cyclic AMP production in fragile X syndrome: cytogenetic and molecular correlations. Pediatr. Res. 38, pp. 638-643.

Chadwick W., *et al.* (2024). A novel combination treatment for fragile X syndrome predicted using computational compounds. Brain Communications, 6(1), pp. 1-17.

Ciecko-Urban J., Fanselow E., Barth A. (2015) Neocrotical Somatostatin Neurons Reversibly Silence Excitatory Transmission via GABAb Receptors. Current Biology, 25(6), pp. 722-731.

Kourdougli N. *et al.*(2023). Improvements of sensory deficits in fragile X mice by increasing cortical interneuron activity after the critical period. Neuron, 111(18), pp. 2863-2880.

Results:

Due to the relative size of the data collected and the groups included a statistical analysis could not be performed at the time on each group present.

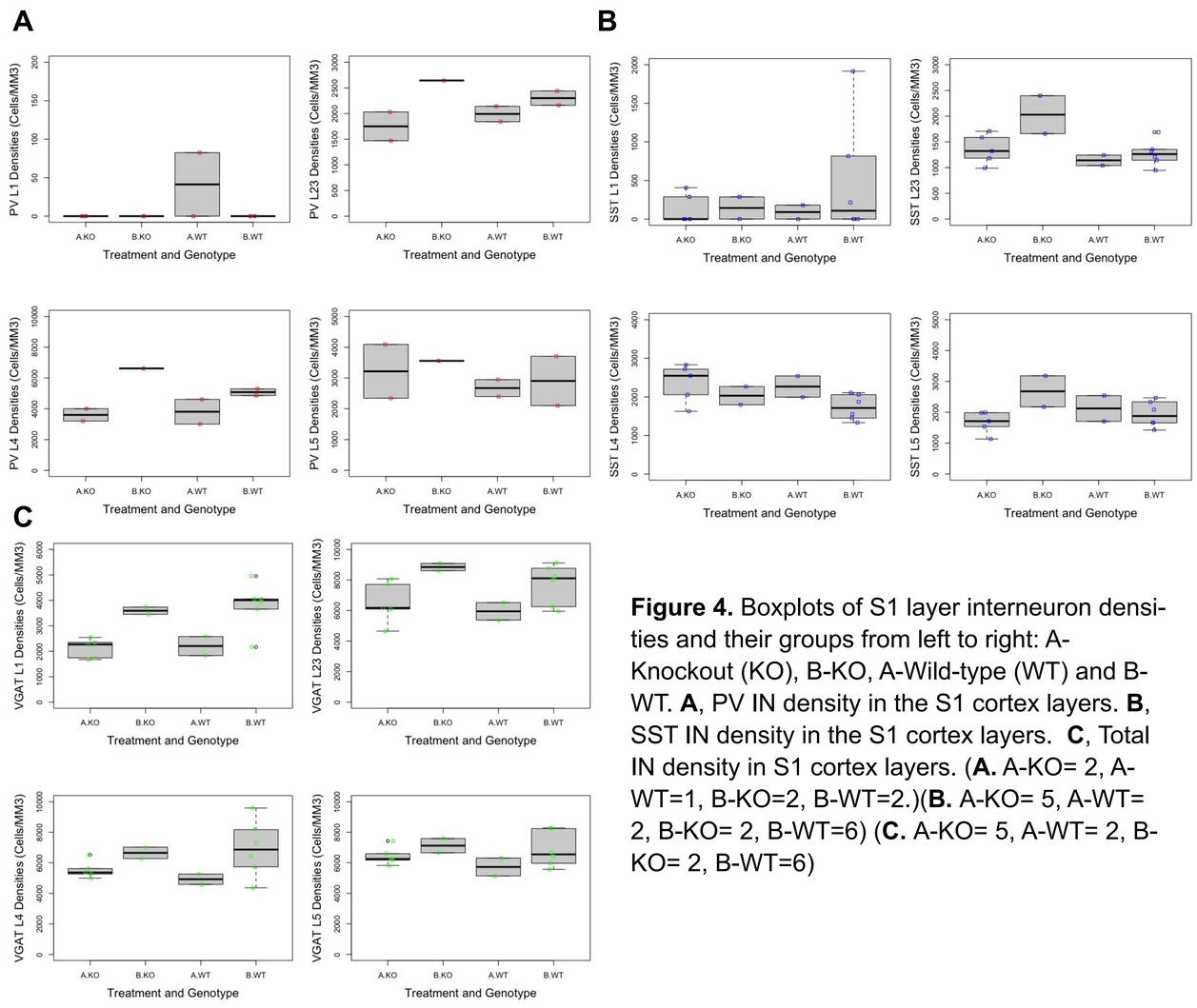

Figure 4. Boxplots of S1 layer interneuron densities and their groups from left to right: A-Knockout (KO), B-KO, A-Wild-type (WT) and B-WT. **A**, PV IN density in the S1 cortex layers. **B**, SST IN density in the S1 cortex layers. **C**, Total IN density in S1 cortex layers. (**A.** A-KO= 2, A-WT=1, B-KO=2, B-WT=2.)(**B.** A-KO= 5, A-WT= 2, B-KO= 2, B-WT=6) (**C.** A-KO= 5, A-WT= 2, B-KO= 2, B-WT=6)

PV,SST and VGAT Data:

- PV data presented treatment B specific increases to PV-IN density in layers 23 and 4, but no changes in Layer 1 or 5.

-SST data showed separate trends among layers. Layers 23 data showed a treatment B specific increase in SST-IN density, however, a decrease in SST-IN density was seen in L4. No trend was noted for L5 or L1.

-VGAT data showed a specific treatment group B increase in total IN density across all layers regardless of genotype.

Discussion:

PV and SST

FXS mice show lower levels of PV interneurons present in the somatosensory regions such as the S1 cortex (Kourdougli N. *et al.*, 2023) The data show a possible elevation of PV density due to treatment B. However, more data is required to establish this trend.

SST-Ins control cortical networks and excitatory population dynamics, suggesting alterations to this population occurs in fragile X syndrome (Ciecko-Urban J. *et al.* 2015). Lack of data mean results allow for no clear conclusions to be made among the layers of the S1 layers between groups. This requires more data collection to understand either treatment A or B's effect on SST densities.

VGAT

The VGAT data provides an overview of how total interneuron density is effected by the different treatment groups and genotypes.

Data showed treatment group B having a higher total interneuron density across all layers regardless of genotype. Interestingly, it also showed no effect of genotype with similar densities seen in both WT and KO. These findings could suggest a recovery of interneuron density in treatment group B.

Figure 5. Summary of trends within interneuron data. Created in Biorender.

Conclusions and Future Research:

- Data suggest treatment group B overall has higher levels of interneuron density than A with no effect of genotype
- More data collection surrounding this is important to discover BPN14770 effect on interneuron subtypes
- Future research should focus on: Increased IN relation to FXS symptom improvement, Dosage effect of BPN14770 on interneuron populations, potential BPN14770 side effects.

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This project was carried out at Booker lab under the guidance of Sam Booker at the centre for Brain Discovery sciences. I would like to further thank Anna Sumera for the help provided during the project.