Homeostatic Regulatory Mechanism in a Mouse Model of Fragile-X Syndrome

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Introduction

Fragile-X Syndrome, or FXS, is the most common inherited learning disability and the leading single-gene cause of autism.[1] It results from a mutation in the FMR1 gene situated on the X chromosome, which prevents the production of FMRP, a protein crucial for brain development. This causes behavioural and cognitive impairments.[2]

Aim of the Study

The study aims to analyze how a lack of FMRP in a knockout mouse model of FXS will affect the:

- Neurovascular coupling - the process linking neuronal activity to blood flow; FMRP deficiency may impair this coordination, reducing the brain's ability to meet metabolic demands.
- Homeostatic regulation of blood vessel physiology - mechanisms that stabilize neural circuit activity; loss of FMRP may disrupt this balance, leading to abnormal signaling and plasticity.

Methods

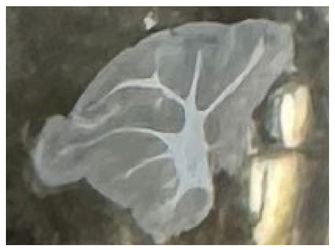
In total, 52 mouse cerebellum were extracted, providing 73 30-microns slices which were used for analysis:

- 8 slices of wild type (WT) male
- 30 slices of wild type (WT) female
- 5 slices of FXS male
- 30 slices of FXS female

The slices were then prepared for the immunofluorescence technique by binding the target protein to a primary antibody and the latter to a fluorescent secondary antibody.

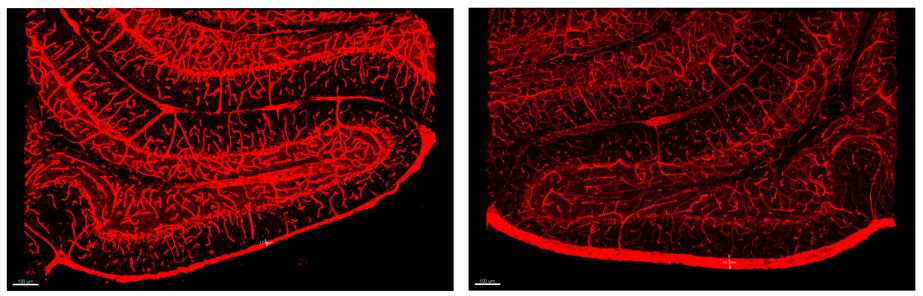
To target vascular proteins, 4 different antibodies were used:

- Anti-GFAP
- Anti-Lectin
- Anti-Collagen
- Iso-Lectin



The next step consists of mounting the cerebellum slices onto glass slides to prepare them to go under the confocal microscope.

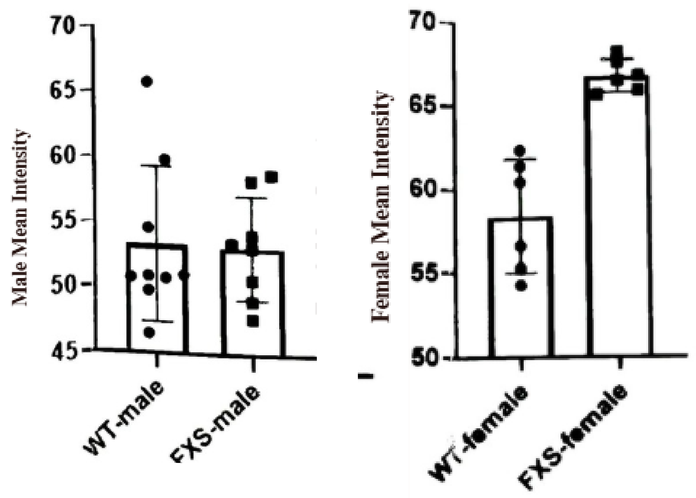
The slices were examined under a confocal microscope and analyzed with FIJI Imagej.

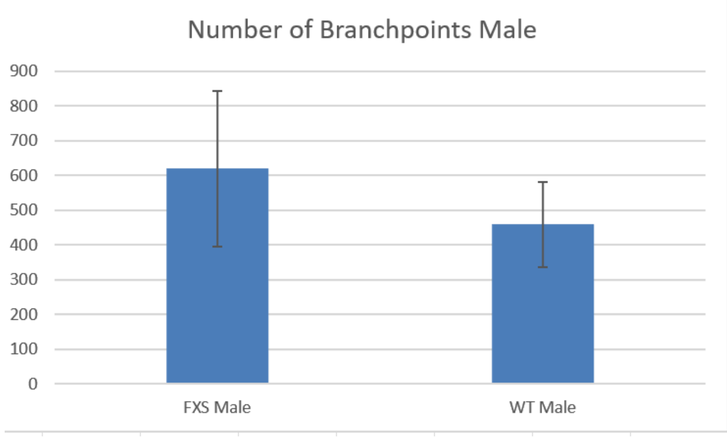


WT FXS

Images of female cerebellum using anti-collagen antibody

Results





NON SIGNIFICANT DIFFERENCES BETWEEN WT AND FXS

- Blood vessel length
- Number of branches
- Vascular density in males
- Vascular density in females (p value = 0.076)
- Number of endpoints
- Average branch thickness
- Number of branch points in females
- Mean vessel intensity in males
- Molecular and granular layers

SEX SPECIFIC SIGNIFICANT DIFFERENCES BETWEEN WT AND FXS

- Number of branch points in males (p value = 0.030)
- Mean vessel intensity in females (p value = 0.0002)

Conclusion

Preliminary results show that no significant difference between WT and FXS exist unless sex is considered among the variables measured. The exploratory analysis should be continued, and more variables should be measured in order to identify and quantify the visual differences. Sample size for the males would need to be expanded.

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References

[1] Cleveland Clinic. "Fragile X Syndrome: Diagnosis, Symptoms & Treatment." Cleveland Clinic, Cleveland Clinic, 7 Feb. 2024, my.clevelandclinic.org/health/diseases/5476-fragile-x-syndrome.
[2] National Institute of Child Health and Human Development. "What Causes Fragile X Syndrome?" Http://Www.nichd.nih.gov/, Dec. 2016, www.nichd.nih.gov/health/topics/fragilex/conditioninfo/causes.