Role of FMRP in regulating the tonotopic distribution of C-fos expression levels in the LSO, AVCN, and MNTB

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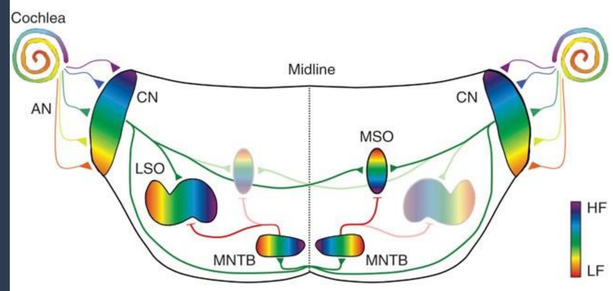
Introduction

- Fragile X Mental Retardation Protein. is important for normal brain development and function. It is encoded by the FMR1 gene and is involved in regulating the translation of other proteins in the brain.
- Mutations in the FMR1 gene can lead to Fragile X Syndrome, a genetic disorder that can cause intellectual disability, behavioral and learning challenges
- The tonotopic axis refers to the organization of sound frequency along a specific axis in the auditory system, important for the processing of sound information.



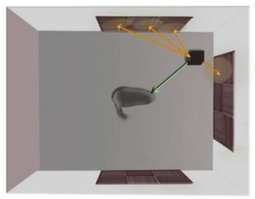
- Preliminary data suggest that FMRP is necessary for maintenance of the gradient of channels across the tonotopic axis and are consistent with a role for FMRP as a repressor of protein translation.
- Tonotopicity may be required for accurate encoding of stimulus features. A disruption of this gradient potentially occurs in Fmr1 animals and degrades processing of this information.

Research Question - Does FMRP play a role in the rapid experience-dependent expression of C fos along the tonotopic axis of LSO, AVCN and MNTB?

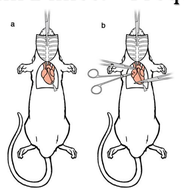
Hypothesis - In WT mice, C fos will be expressed along a tonotopic gradient in the auditory regions of interest. In Fmr1 mice, which lack FMRP, the tonotopic distribution of C fos expression will be flattened relative to WT controls.

Methodology

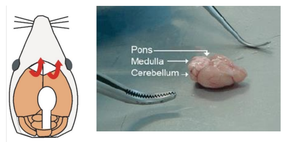
Measure C-fos expression levels in LSO, AVCN and MNTB after 5 minutes of acoustic stimulation at a specific frequency WT and Fmr1 mice. Frequencies tested – 8kHz, 16kHz and 22kHz



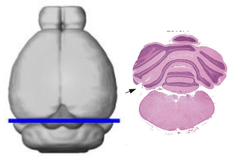
5 mins sound exposure @ 8/16/22 KHz
2 hours for C fos expression
Sound attenuating Chamber



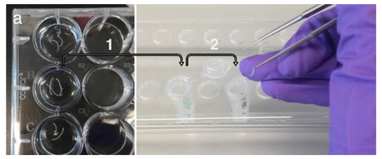
Animal euthanasia using pentobarbitol and perfusion



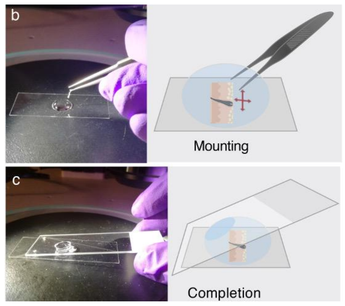
Brain dissection and processing

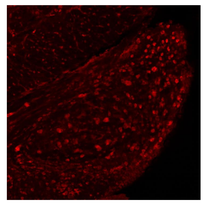


Brain sectioning – coronal
Thickness - 70um

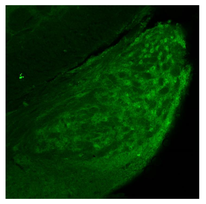


C fos Staining
1. Blocking
2. Primary C fos antibody
3. Secondary fluorophore antibody
4. Nissl Staining





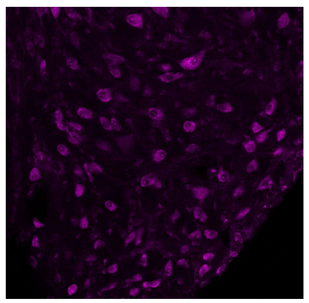
Nissl Staining



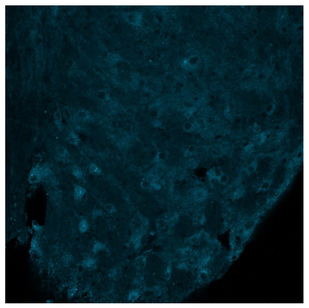
C fos Staining

Imaging using Confocal microscopy

Results

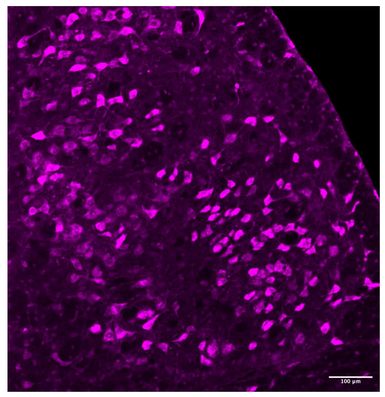


a)

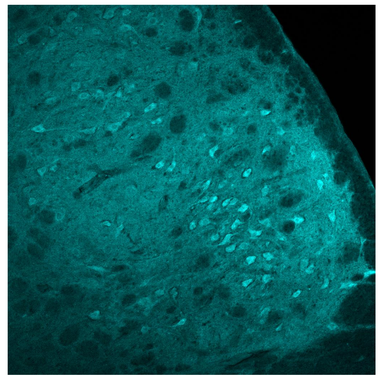


b)

AVCN Male wildtype 16Khz
a) Nissl Staining
b) Band of C fos expression



Nissl Staining



Band of C fos expression

LSO Female wild type 16Khz

Conclusion

- C fos expression protocol is working as expected
- We expect to be able to visualize and quantify differences in C fos expression in wildtype and Fmr1 mice with our methodology
- With present data, we see different bands of C fos expression in auditory regions of wild type mice at different frequencies – correlates with hypothesis

Future Directions

- Optimize the methodology for visualization and quantiification of C fos expression
- Collect larger sample size for each frequency
- Broadly
  - Investigate the functional consequences of altered tonotopicity
  - Understand the relationship between FMRP and tonotopicity and how this interaction may contribute to auditory processing deficits in individuals with Fragile X syndrome

Acknowledgements

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