Examining The Influence of Fragile X Syndrome on Tonotopic Precision in Mice

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Abstract

Fragile X Syndrome is a condition resulting from a genetic mutation of the FMR1 gene. As a result, learning disabilities, cognitive impairments, and other developmental illnesses may arise. However, auditory impairments are the most prevalent of symptoms that are associated with this mutation. The symptoms of Fragile X Syndrome are severely underexplored, thus through our research endeavors we are attempting to examine tonotopic precision in the auditory brainstem of mice that lack the FMR1 gene. We have hypothesized that the tonotopic precision is reduced in FMR1 Knockout mice, as that is consistent with the hyperactive nature of the organisms as well as the auditory phenotypes of this disease. In order to carry out this experiment, we have utilized c-fos, an early gene product that can be used as a marker of cell activation in order to anatomically map the response of sound stimulation. We then compared the c-fos band ratio of the knockout mice (those lacking an FMR1 gene) with the wildtypes (healthy mice) in order to determine if the tonotopic precision is reduced. The tonotopic precision is typically determined through extrapolating the ratio of the width and the ratio of the area of neuronal activity in particular regions while the mice are exposed to auditory stimulation. Our data so far concludes the fact tonotopic precision is significantly less in the knockout mice than in the wildtype mice. Such conclusions were shown when the mice were exposed to 8 kilohertz as well as 16 kilohertz of sound.

Introduction

A genetic mutation by the name of Fragile X Syndrome is caused by the absence of the FMR1 gene expression(Chen *et al.* 2001). This syndrome is the leading genetic cause of autism spectrum disorders in children (Chen *et al.* 2001). The FMR1 gene is an x-linked gene, meaning that it is only present on the X (maternal) chromosome. It is typically expressed in the ovaries, testes, and brain and provides instructions for the creation of a protein known as FMRP. While examining a healthy brain, earlier studies have indicated that FMRP are usually strongly expressed in sensory-associated regions of the brain, the hippocampus, cerebellum, cerebral cortex, olfactory bulb, nucleus basalis, and the corpus callosum. Thus, a loss of the FMRP results in the abnormal structure and function of neurons. Patients with this syndrome exhibit phenotypical side-effects such as learning and memory deficits, sensory and motor dysfunction, as well as social ineptitude. Additionally, FMRP are also prevalent in auditory neurons, suggesting that Fragile X Syndrome may also cause auditory impairments (Chen *et al.* 2001). Past studies conducted on FMR1 knockout mice have suggested that this syndrome is consistent with reduced dynamics in ion channel regulation of auditory brainstem neurons and altered synaptic connectivity. However, the brain mechanisms underlying the phenotypes of Fragile X Syndrome are poorly understood.

Our experiment entails performing auditory stimulation of varying frequency on 2 to 3 month-old mice using speakers and a sound-insulated chamber. Prefusion of the mice, dissection, and sectioning of the brains were conducted. Subsequently, primary and secondary antibody treatments were performed in order to detect c-fos activity in the brains of the mice. C-fos is a proto-oncogene which is commonly used as a marker of neuronal activation, including in FMR1 knockout mice (Rogers *et al* 2017). Exposure to auditory stimulation typically induces c-fos expression, and thus we used this indicator in order to examine whether neuronal activity is present in certain parts of the brain as well as the activity's tonotopic precision.

Scientific Question & Hypothesis

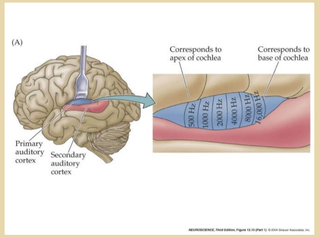
Scientific Question: How are tonotopic precision altered in mice that exhibit Fragile X Syndrome (FMR1 Knockout) as exhibited by early gene expression (c-fos)?

Hypothesis: We hypothesize that the tonotopic precision is reduced in FMR1 Knockout mice, as that is consistent with the hyperactive nature of the organisms as well as the auditory phenotypes of this disease.

Method

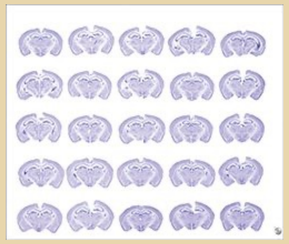
This experiment began with placing 2 to 3 months old mice in a sound-insulated glass chamber and auditory stimulating them using speakers for approximately 1.5 hours. The mice were separated into experimental groups: FMR1 Knockout mice (those exhibiting Fragile X syndrome) and wildtype mice (healthy mice). The mice were stimulated with 8 kilohertz and 16 kilohertz of sound. Subsequently, the mice were immediately perfused, followed by dissections of their brains. The brains were then coronally sectioned into 40 micrometer sections. The consequent sections were immunostained with c-fos and NeuroTrace and mounted for viewing under a confocal microscope. The confocal microscope imaging allowed us to use ImageJ software to quantify the c-fos-labelled cells and calculate a ratio of c-fos band ratio as shown by the two experimental groups after being administered two variables of auditory stimulation.

Image 1: Depicting The Tonotopic Areas Where Sound is Transmitted in the Cochlear Nucleus



Purves, D. (1970, January 1). Figure 13.14, [the human auditory cortex. (a)...]. - neuroscience - NCBI bookshelf. Neuroscience. 2nd edition. Retrieved March 2, 2022, from https://www.ncbi.nlm.nih.gov/books/NBK10900/figure/A920/

Image 2: Sample of mounted mice brain sections



Slamdot, I. (n.d.). Embedding & Sectioning . Neuroscience Associates. Retrieved March 2, 2022, from https://www.neuroscienceassociates.com/services/embedding-and-staining/

Results

Our data so far suggests the fact tonotopic precision is less in the knockout mice than in the wildtype mice. The FMR1 Knockout mice who were auditory stimulated at 8 kHz had an approximate c-fos band ratio of 0.2 in comparison to a 0.1 band ratio from the wildtype mice. Similarly, the FMR1 Knockout mice with an auditory stimulation of 16 kHz has a c-fos ratio of about 0.6, while the wildtype mice had a ratio of 0.2-0.3.

Image 3: Sample of confocal microscope imaging of stimulated tonotopic areas of the knockout mice brains

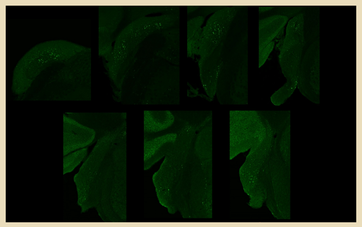
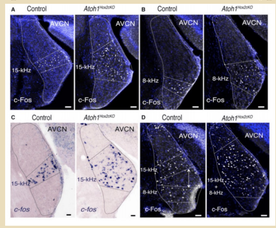


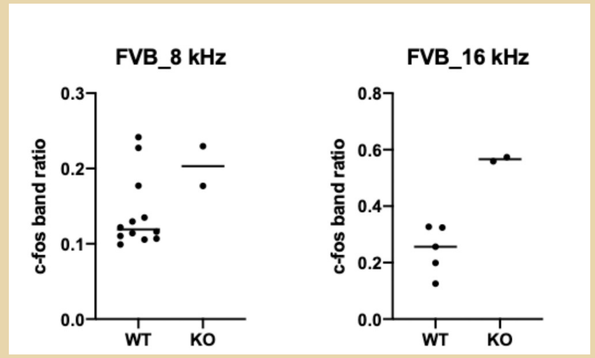
Image 4: Confocal microscope imaging of stimulated tonotopic areas of the knockout & wildtype mice brains



Karmakar K, Narita Y, Fadok J, Ducret S, Loche A, Kitazawa T, Genoud C, Di Meglio T, Theirry R, Baeclo J. Luthi A, Jijli FM. 2017. Hox2 Genes Are Required for Tonotopic Map Precision and Sound Discrimination in the Mouse Auditory Brainstem. Cell Reports 18:185-197.

Data

Figure 1: Data results of 8 and 16 kHz Auditory Stimulation of Wildtype and Knockout Mice



Conclusion & Discussion

Our results supported that tonotopic precision is decreased by Fragile X Syndrome. This can be proven by the observation that the tonotopic c-fos expression of knockout mice was broader than that of wildtype mice. These results allow us to deduce that our hypothesis was supported and that tonotopic precision is reduced in FMR1 Knockout mice. This broadening of expression may be a result of an excess of excitatory neurons or their connectivity that lead to increased firing rates, and thus abnormal sound localization cues.

Our study is still currently ongoing and thus, we will continue to extrapolate data in order to analyze whether and how tonotopic precision is affected by this genetic mutation and to further develop and support our results,

Citations

Chen, L., & Toth, M. (2001). Fragile X mice develop sensory hyperreactivity to auditory stimuli. Neuroscience, 103(4), 1043–1050. https://doi.org/10.1016/s0306-4522(01)00036-7

Rogers, T. D., Anacker, A., Kerr, T. M., Forsberg, C. G., Wang, J., Zhang, B., & Veenstra-VanderWeele, J. (2017). Effects of a social stimulus on gene expression in a mouse model of fragile X syndrome. Molecular autism, 8, 30. https://doi.org/10.1186/s13229-017- 0148-6