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Analysis of Ultrasonic Vocalizations in a Rat Model of Fragile X Syndrome

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Abstract

Fragile x syndrome (FXS) is a leading known inherited form of autism and autism spectrum disorder (ASD) with deficiencies in social communication and sensory processing. It is caused by a mutation in the fragile x mental retardation gene (Fmr1). The purpose of this research is to determine if the mutations associated with ASD recapitulate the core communicative deficit features of this disease in rat models. Reproducing and analyzing these features in rat models can shed more light on the pathophysiology of ASD.

To address this, social communication, in the form of ultrasonic vocalizations (USVs) were collected and analyzed from control (wild type) and Fmr1 KO rats from different rearing environments and call-inducing social conditions. Analysis of these USVs suggests that a complex interplay between genes and rearing environment influence the manifestations of specific communicative deficit features of FXS.

Materials and Methods

GENOTYPING

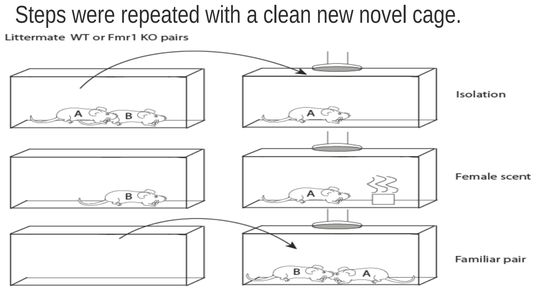
- USVs were obtained and analyzed from two cohorts
  - Cohort I: aged-matched WT male rats bred at Charles Rivers Laboratories (CR) and homozygous Fmr1-KO male rats bred at SAGE Laboratories
  - Cohort II: littermate WT and Fmr1-KO male rats, bred at UB Lab Animal Facility from heterozygous Fmr1-KO females and WT males

COLLECTION OF USVs

- All rats were exposed to 3 different call-inducing conditions: isolation, female scent, and familiar pair.
- USVs were collected blind to genotype from both WT and KO rats on the same days
- 2 minute USVs were recorded for each condition, using the Avisoft-SASLAB Pro software and equipment.
- USV analysis was conducted blind to genotype and condition via Adobe Audition and Raven software

STEPS FOR USV COLLECTION

1. A rat is separated from its WT littermate and placed alone in another novel cage environment with bedding (record isolated-induced USVs).
2. The gauze soaked with female urine is introduced (record female scent-induced USVs).
3. Gauze is removed, and a littermate is reintroduced (record familiar pair-induced USVs).
4. Steps were repeated with a clean new novel cage.



Results

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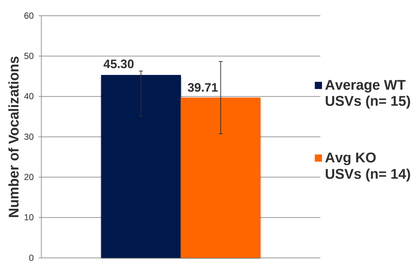

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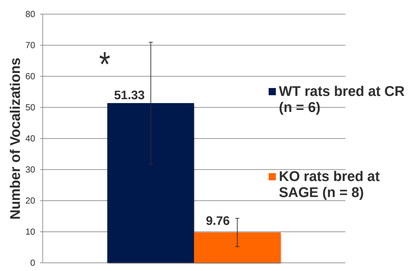

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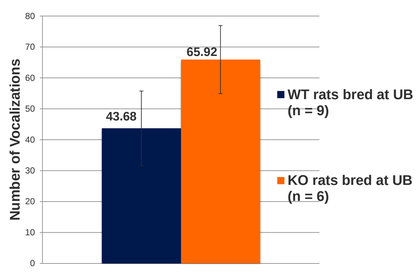

The effect of different rearing environments on average number of vocalizations produced by WT and KO rats. There was no significant difference between genotype on call rate ($p = 0.68$, Fig. 1). Within cohort I, WT (CR-bred) significantly vocalized more than KO (SAGE-bred) rats (*$p = 0.02$, unpaired t-test, Fig. 2). Conversely, an opposite trend was in cohort II with (UB-bred) WT and KO rats, which did not reach significance ($p = 0.21$, Fig. 3).

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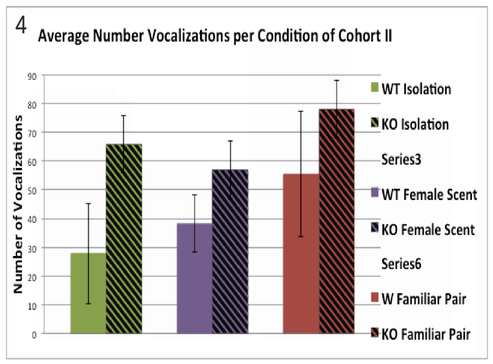

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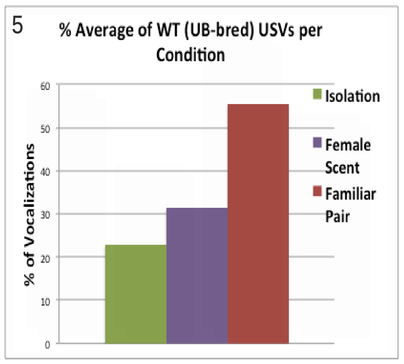

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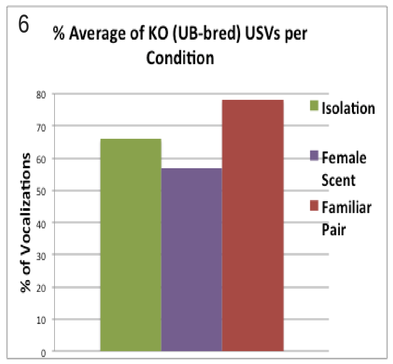

The effect of socially motivated conditions on call-rate in WT and KO rats. Within cohort II, KO rats vocalized more than WT rats across conditions (Fig 4.). Yet, WT rats tend to vocalize more during the familiar pair and female scent conditions than in isolation (Fig. 5). Contrarily, KO rats vocalized relatively the same amount during each socially motivated conditions (Fig 6).

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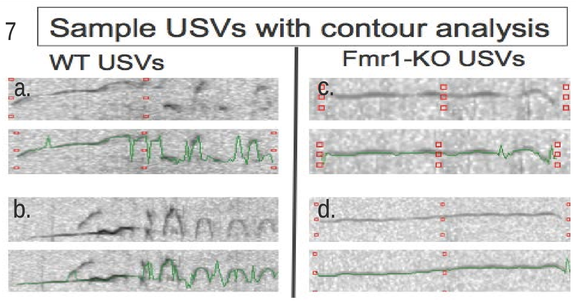

Observable complexity differences in USVs from WT and KO rats

Fig. 7a: USV from a UB-bred WT rat
Fig. 7b: USV from a CR-bred WT rat
Fig. 7c: USV from a UB-bred KO rat
Fig. 7d: USV from a SAGE-bred KO rat

WT rats appear to produce more complex and varied USVs than KO rats.

Call structure was analyzed by RAVEN software's average peak contour frequency function (contours shown in green).

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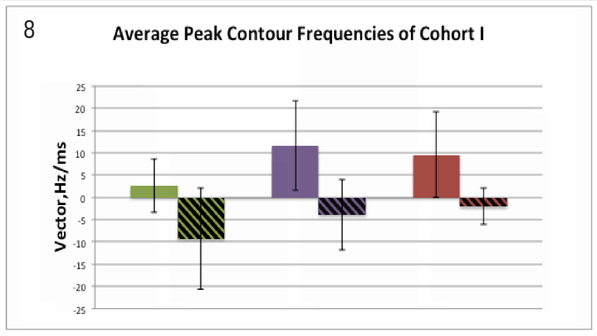

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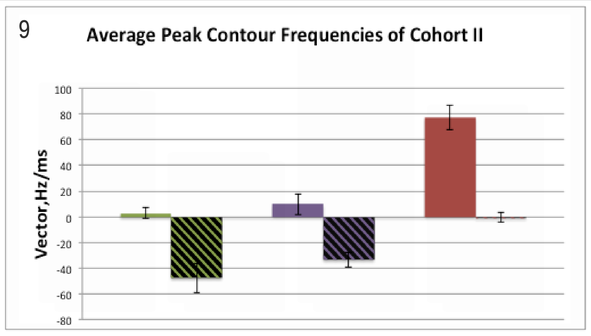

WT Isolation | KO Isolation | WT Familiar Pair | KO Familiar Pair | WT Female Scent | KO Female Scent

Analysis of call structures in cohort I and II. Differences in average PFC slope between WT and KO rats suggests genotypic-specific call structures. Both genotypic and social condition related differences in call structure were conserved across rearing conditions. (Fig. 8 and 9)

Conclusion & Future Work

Conclusion

- Different rearing environments effect the average number of USVs produced by WT and KO rats.
- Further breakdown of the average number of USVs collected from WT and KO rats during each social condition reveal genotype-specific trends.
- Call structure, measured by average PFC slope of USVs, reveal genotype specific trends.
- PFC analysis may provide a means for objectively measuring and analyzing core communicative deficits in rats model of FXS.

Future Work

- Investigate the effect of parental genotype on the number and types of USVs produced by offspring.
- Reduce the background noise in USV files to allow for more accurate and precise contour analysis
- Classify USVs into specific call groups such as up-sweep trills, down-sweep trills, step, step-trill, and complex for additional analysis of call structure.
- Improve USV collection apparatus by including a glass partition in the familiar pair condition in order to collect and analyze vocalizations solely from rat of interest
- Repeat experiment with more WT and KO rats

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