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## ABSTRACT

Core symptoms of Autism Spectrum Disorders (ASD) include deficits in social/communicative behaviors, and repetitive/stereotyped behaviors [1]. Mouse models are a highly established paradigm used to study the phenotypic deficits that result from various inducible genotypic or environmental risk factors for ASD. Previous studies have demonstrated a link between maternal immune activation (MIA) and ASD-like behaviors in mouse models [2]. In this model, the maternal immune system is activated during pregnancy by injecting the viral mimic poly(I:C). The resulting offspring are phenotyped and analyzed with regards to their communicative behaviors [3].

Previous studies have demonstrated that pups born to mice with immune activation produce fewer ultrasonic vocalizations (USVs) in testing than do their saline-injected counterparts. It has also been found that MIA produces offspring with hallmark signs of ASD: social deficits and stereotyped, repetitive behaviors [2]. In the present study, the MIA model is assessed as a mouse model for ASD with a focus on the shapes of the USVs they produce. Some significant differences were seen in call types between wild-type and MIA animals that are indicative of differing levels of complexity in their communicative behaviors. These results provide insight into the impact of models like MIA as an environmental risk factor for autism and a need for further research on ways to improve maternal and infant health outcomes.

## AIMS AND HYPOTHESIS

- Our goal is to analyze the structural features of ultrasonic vocalizations (USVs) from mice born to dams exposed to Poly I:C, and from control mice born to dams that received saline.
- It was expected that some differences in number or complexity of different call types would be observed between groups.
- Injection dosage, methods to reduce prenatal stress, and gestational timing of injections were based on literature, pilot research, and prior data from a similar study which failed to show significant behavioral effects among offspring.
- Long-term goals include using the translational nature of this data to understand broader implications for human maternal health and risk-factors for ASD in offspring.

## METHODS

USVs were detected automatically using an ultrasonic microphone (B&K) and acoustic analysis software (Adobe Audition). For each trial, female bedding was spread on the bottom of a clean cage and male test subjects were placed in that cage for a habituation period. Then, a female mouse was introduced into the cage. It was expected that the male would vocalize to the female using a variety of established mating calls, and that female vocalizations would be negligible (thus vocalizations recorded and analyzed are male-only). Recordings were taken over 5 minutes, and vocalizations were automatically logged through our computer program. Using previously established call types, the vocal repertoire of each mouse was individually and manually analyzed for shape and duration into categories developed by Heckman et al. (2017) [4].

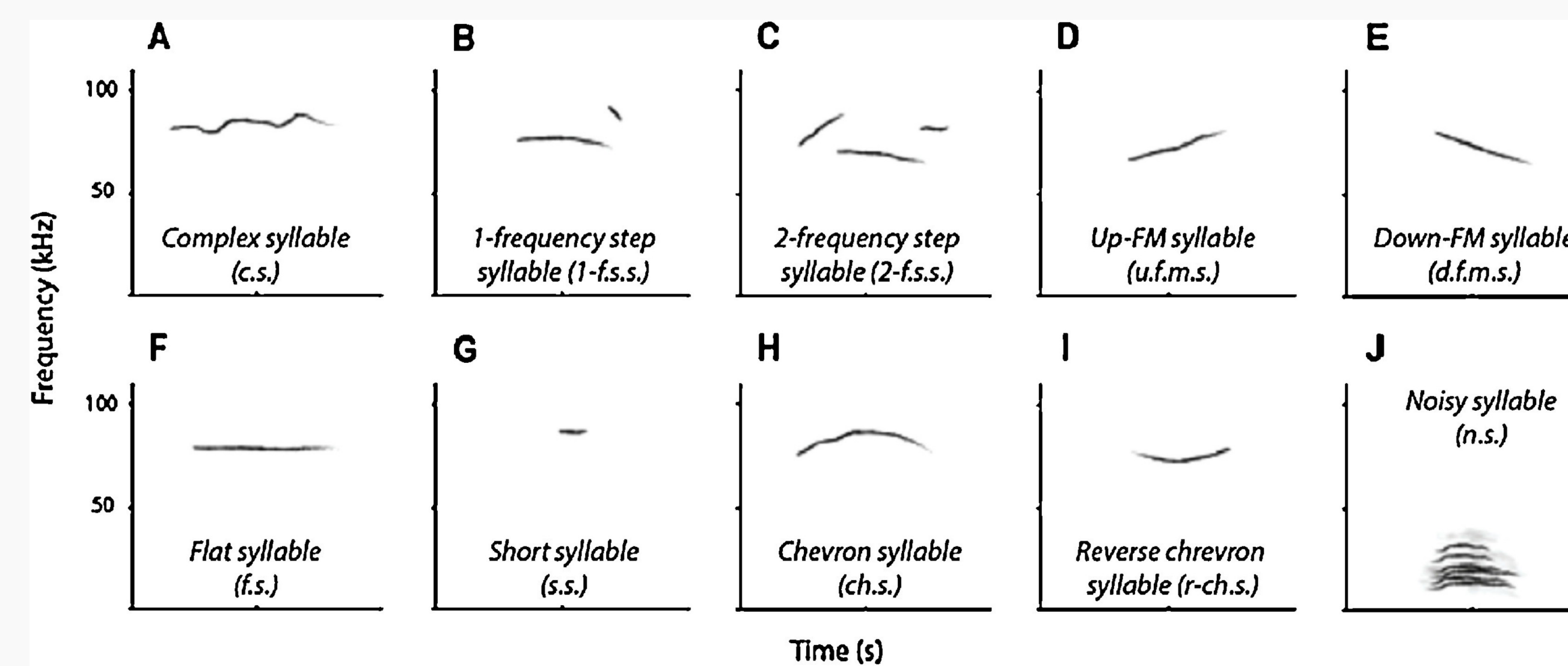


Fig. 1. Mouse USV subtypes, adapted from Heckman et al. (2017). In the current study, B and C were defined as “Frequency Jumps” and J was defined as “unstructured”. An additional category called “modulated” was also included. The final ten categories used to analyze and label USVs were therefore: short, flat, up, down, modulated, complex, unstructured, frequency jumps, chevron, and reverse chevron.

## RESULTS

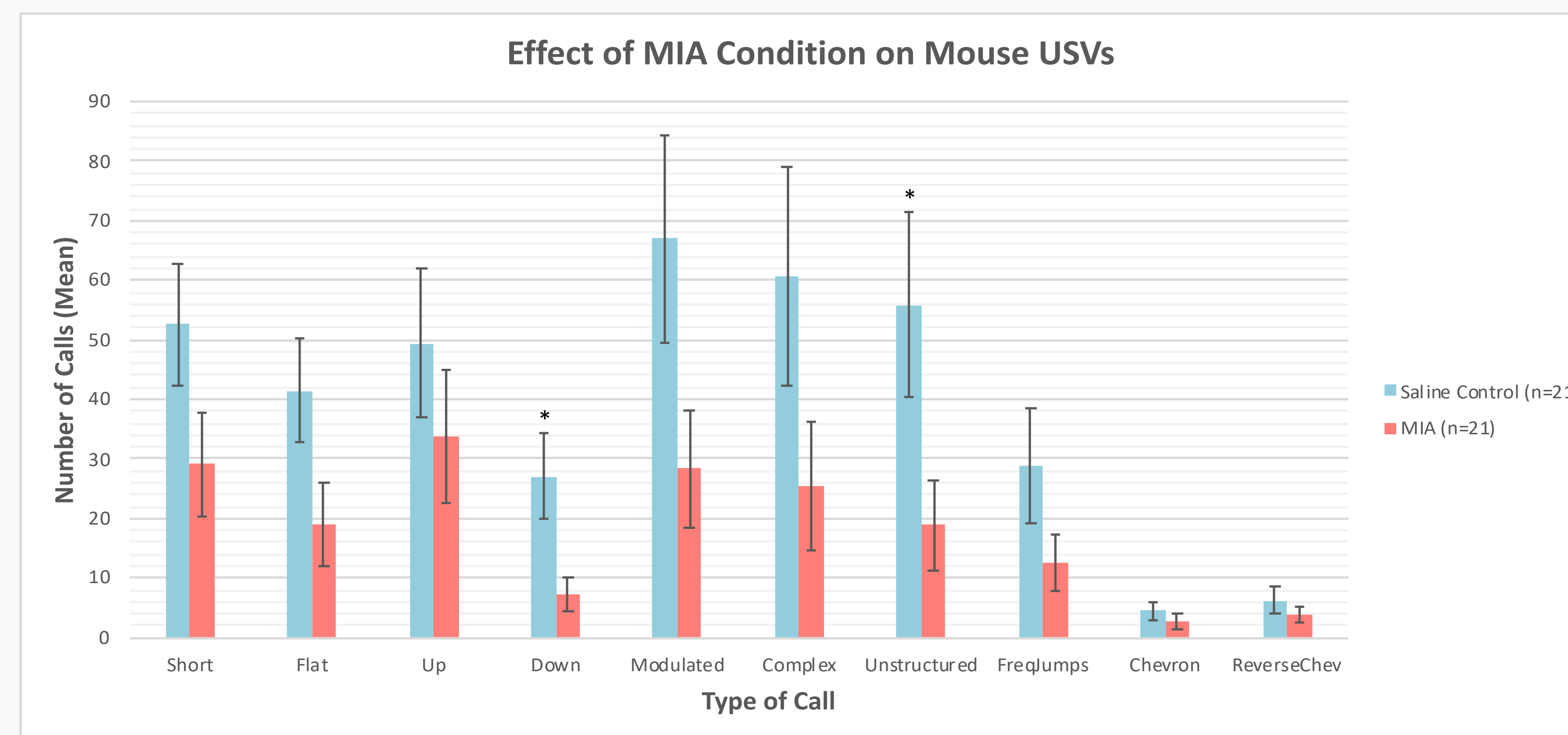


Figure 2. Effect of MIA Condition on Mouse USVs using ANOVA test. The saline control represents wild-type mice that were treated with saline solution. MIA represents mice born from MIA mothers. A test of between-subject effects found a significant difference between wild-type and MIA animals ( $F [1,42] = 4.306, p = .044$ ). The star (\*) symbol demarcates conditions where a significant difference was observed between wild-type and MIA animals with regards to call type. The unstructured ( $p=.04$ ) and down ( $p=.014$ ) call types were seen significantly more in wild-type mice, showing that MIA has some degree of effect on communication in mice.

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## DISCUSSION

The test condition had an effect on the overall differences between the MIA and control conditions, with significant differences in certain categories of mouse calls. In all cases, control mice emitted more calls than MIA mice, and this was significant for the categories “unstructured” and “down.” It is worth noting that the “unstructured” call is referenced as “noisy” in other mouse USV studies. However, in general, the exact social meaning of the different call types is not well understood. This makes the differences observed in these two particular categories of significant interest. Future studies should focus on why MIA mice used as an ASD model might show specific reductions in these types of vocalizations. We hypothesize this could be due to the higher complexity of these call types, but confirmation would require further study.

## FUTURE DIRECTIONS

The current results may lead to further studies that could inform minimization of prenatal factors that increase ASD risk for offspring. For example, additional studies on the critical parameters (timing, degree, key inflammatory factors) associated with maternal immune activation and resulting behavioral deficits could inform screening and/or preventative measures during pregnancy. In addition, further study of the specific deficits observed in certain categories of vocalizations should be used to assess relevance to communicative aspects of ASD in general, for example by ascertaining whether similar specific patterns of vocalization anomalies are seen in other mouse ASD models, and by conducting further assessment on the particular social meaning of these particular calls. Such studies could substantially enhance our understanding of the communicative aspects of ASD in humans.

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