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Sex-Specific Vocalization Impairments in A53T α-synuclein Mutant Mice

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Abstract

Parkinson's Disease is a neurodegenerative disorder that is characterized by both motor and non-motor symptoms, including vocalization deficits. Among the various causes of Parkinson's Disease, a mutation in the α-synuclein gene is a significant contributor. In this paper, we analyze a dataset containing acoustic vocalization measurements from both A53T mutant and wild-type mice, comparing WT vs. A53T within males and within females for simple vocalizations. The dataset included parameters such as duration, maximum power, and frequency ranges of simple vocalizations. Mutant mice did not exhibit significant differences in the duration of simple vocalizations compared to the control group. However, they showed a significantly broader frequency range in simple calls in females, with no measurable difference in males. In addition, female mutant mice exhibited higher maximum acoustic power than female controls, while no differences were observed in males. These findings indicate that the A53T mutation in the α-synuclein gene selectively alters frequency-related aspects of simple vocalizations, with additional effects on vocal intensity that are more pronounced in females. This suggests that vocal communication deficits in this Parkinson's Disease model may be sex-dependent, with frequency range providing the most sensitive marker of genotype-related differences.

Keywords: Parkinson's disease (PD), vocalization, α-synuclein, sex differences, neurodegeneration, rodent models, bioacoustics

1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder of movement that has features of cognitive impairment, autonomic dysfunction, sleep disorders, hyposmia, and depression (Poewe et al., 2017). PD affected a large portion of the population, approximately 6.1 million people worldwide (Bloem et al., 2021). Although the mechanism has not yet been fully understood, the prevalence of the disease has risen rapidly over the past few decades, and the personal impact of the

disease is immense. PD includes two types of symptoms: motor and non-motor. Motor symptoms refer to the movement-related symptoms, including slowness of voluntary movements with progressive reduction in speed, muscular rigidity, and postural instability (Hughes et al., 1992).

On the other hand, non-motor symptoms are not related to movements and can appear before motor symptoms do. The non-motor symptoms include a lack of emotional involvement, sleep problems, loss of smell and taste, mood disturbances, fatigue, and pain (Pont-Sunyer et al., 2015). Genetic mutations are among the most significant contributing factors to the various causes of PD. (Bloem et al., 2021.)

Among them, α-synuclein is particularly closely linked to PD through mutations such as A53T, a mutation in the Alanine residue. This mutation is known to be associated with many detrimental symptoms of PD (Choi et al., 2008; Spira et al., 2001). The mouse model of PD with the A53T mutation develops numerous sensorimotor and synaptic impairments, followed by age-associated cognitive and motor deficits (Paumier et al., 2013). Furthermore, A53T α-synuclein in astrocytes contributes to initiating the damaging process that leads to neuron death (Gu et al., 2010).

Because α-synuclein aggregates disrupt dopaminergic and brainstem circuits, they can impair fine motor behaviors such as respiration–phonation coupling and vocal fold control (Rektorová et al., 2012; Ciucci et al., 2007). In humans, this manifests as prosodic flattening, reduced articulatory precision, and vocal fold atrophy, which may also contribute to impaired swallowing (Bocklet et al., 2011; Rektorová et al., 2012; Yiu et al., 2020). Similarly, rodent models show parallel deficits: α-synuclein transgenic mice exhibit early-onset and progressive impairments in ultrasonic vocalization, including reduced call duration, intensity, and altered spectral profiles that often appear by two to three months of age and precede motor decline (Grant et al., 2014; Grant et al., 2015). Collectively, these findings indicate that α-synuclein overexpression and aggregation disrupt vocal control circuits across species, highlighting vocalization as a sensitive biomarker of Parkinson's disease (PD) (Gnerre et al., 2023).

Measures such as call duration, frequency modulation, and vocal intensity in mice are considered counterparts of human PD vocal deficit symptoms. In mice, changes in call duration match prosodic timing deficits in PD speech (Gnerre et al., 2023; Rektorová et al., 2012), reduced frequency modulation represents the monotone voice and pitch variation of patients (Bocklet et al., 2011; Houle et al., 2024), and lower call intensity mirrors hypophonia or reduced loudness, which are symptoms of PD (Grant et al., 2014, 2015; Ramig et al., 2001).

1.1. Sex Differences in Parkinson's Disease

Epidemiological studies suggest that sex is a crucial factor in PD development. The disease is approximately twice as common in men as in women (Baldereschi et al., 2000; Solla et al., 2012). Women tend to develop PD about two years later than men, likely due to estrogen-related protective factors such as menopause and childbirth (Haaxma et al., 2007). Women are more likely to present with tremors as an initial symptom, associated with a slower progression and milder motor course. By contrast, men tend to exhibit more severe motor symptoms, including speech problems, while women experience more non-motor symptoms such as fatigue, depression, and pain (Santos-García et al., 2023). Longitudinal studies confirm these patterns: men show faster decline in both motor and non-motor aspects, requiring higher medication doses and experiencing greater impairment in daily tasks (Picillo et al., 2022). However, other reports suggest that women with PD sometimes report worse disability, quality of life, and greater anxiety despite physicians observing no major symptom

differences (Abraham et al., 2019). These findings highlight the complex and sometimes contradictory nature of sex differences in PD progression and symptom burden.

1.2. Sex Differences in Vocal Effects

Sex-related differences also extend to vocal impairments in PD. Analysis of speech recordings revealed that while most acoustic measures—such as pitch variability, speech rate, and vowel articulation—are affected by both PD and sex independently, there are sex-specific interactions. For example, females with PD more often produced multiple bursts during plosive consonants, whereas males frequently failed to make a burst, indicating sex-dependent disruptions in articulatory timing and force (Houle et al., 2024). Similarly, Gnerre et al. (2023) showed that emotional expression in speech was impaired in PD patients, particularly women, who demonstrated lower pitch when expressing pleasure and poorer voice quality when expressing fear and anger. Neutral speech, however, showed no sex-related differences. Complementing these findings, Hertrich and Ackermann (1995) reported that women with PD exhibited more irregularities in sustained vowel production, such as shaky pitch and abnormally low sounds, compared to both controls and men with PD. These results suggest that PD affects male and female voices differently, potentially due to inherent differences in vocal cord structure and function.

In this paper, we investigate whether there are sex differences in vocalization phenotypes in the A53T mouse model of Parkinson's Disease (PD). Specifically, we aim to explore distinct differences between male and female vocal expression, a non-motor symptom often observed in PD. To address this, we analyzed an existing vocalization dataset from A53T mutant mice and wild-type controls, focusing on simple vocalizations. Simple calls are defined as those with a constant, non-modulating frequency (Krasko et al., 2021). Because simple calls rely more heavily on basic motor pathways, they may be particularly vulnerable to early disruptions in PD (Holy & Guo, 2005). Thus, simple calls are especially sensitive to alterations in timing and intensity, paralleling hypophonia and prosodic timing deficits observed in PD.

We hypothesize that A53T mutant mice will exhibit sex-specific alterations in simple vocalizations. Specifically, we predict that females will show greater variability and disruption in acoustic parameters than males, consistent with evidence that sex hormones influence dopaminergic circuits involved in vocal motor control (Gillies & McArthur, 2010; Van Den Eeden et al., 2003; Ciucci et al., 2007).

By testing this hypothesis, we aim to clarify how non-motor symptoms manifest across sex, shedding light on potential mechanisms underlying sex-specific vulnerabilities in PD. While the original dataset provides raw acoustic measures of A53T mutant and wild-type mice, it did not explore how these alterations vary by sex. Our analysis builds on this by uncovering sex-specific patterns in simple vocalizations that suggest distinct neural mechanisms and highlight the potential for more precise biomarkers of Parkinson's disease.

2. Methods

The Purdue University Research Repository is the source of this information. This dataset is publicly accessible at: https://purr.purdue.edu/publications/4583/supportingdocs/1. A publicly accessible vocalization dataset from A53T mutant mice and wild-type mice (which served as the control group) was used for our analysis. Our analyses included 7 WT males, 7 GFP+ males, 6 WT females, and 9 GFP+ females. We focused on several key acoustic parameters: Duration, Power Maximum,

and frequency range, comparing WT vs. A53T within each sex for simple calls. To evaluate distributional differences between genotypes (WT vs. A53T) within sex for simple calls, we used R to perform two-sample Kolmogorov–Smirnov (KS) tests. The KS test was chosen because it is a non-parametric method that does not assume normality or equal variances and is appropriate for comparing two distributions. Unlike tests that compare only group averages (e.g., t-test or Mann–Whitney U), the KS test evaluates differences across the entire distribution, making it suitable for detecting shifts in both central tendency and variability of acoustic parameters. We were able to determine whether the observed differences were statistically significant by using the KS test. Results with p-values below 0.05 were considered statistically significant.

3. Results

3.1. Vocalization Duration

This study investigated whether there are sex differences in vocalization phenotypes in an animal model of Parkinson's disease. To explore this question, we obtained the vocalization dataset from A53T mutant mice and wild-type mice (the control group). We focused our analysis on simple vocalizations, which are defined as calls with a constant, non-modulating frequency (Krasko et al., 2021). Examining simple vocalizations by sex is important for understanding whether male and female mice exhibit distinct alterations in vocalization duration.

First, we investigated whether there is a sex difference in vocalization duration by analyzing samples from males and females in the simple vocalization test. The cumulative distribution plots showed no significant difference between male wild-type and male mutant animals (Figure 1, left panel; KS test, p = 0.182). Similarly, female wild-type and female mutant animals also did not differ significantly in their vocalization duration (Figure 1, right panel; KS test, p = 0.176). These results indicate that, when separated by sex, mutant mice do not show altered duration of simple vocalizations compared to wild-type controls.

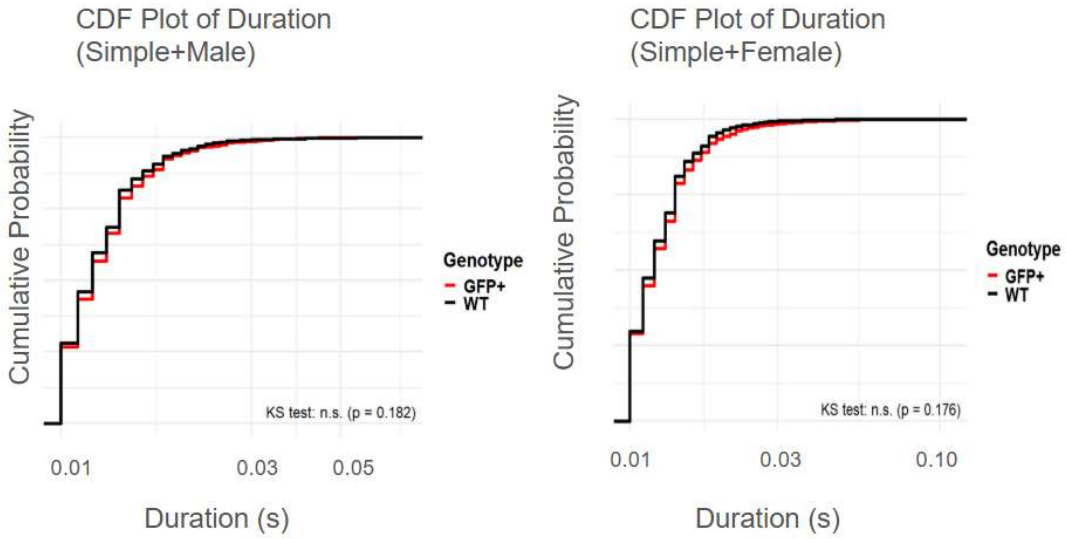

Figure 1: The Biological Principle of Donor-Derived Cell-Free DNA (dd-cfDNA) as a Biomarker.

3.2. Vocalization Frequency Range

Secondly, to determine whether the mutation alters the frequency range of simple vocalizations, we examined male and female mice separately. Male mutant mice did not differ significantly from their wild-type controls (KS test, p = 0.253). In contrast, female mutant mice exhibited a significantly broader frequency range compared to female wild-type controls (KS test, p = 0.0287) (Figure 2).

Taken together, these findings indicate that the A53T mutation increases the frequency range of simple vocalizations in females, while males show no measurable differences.

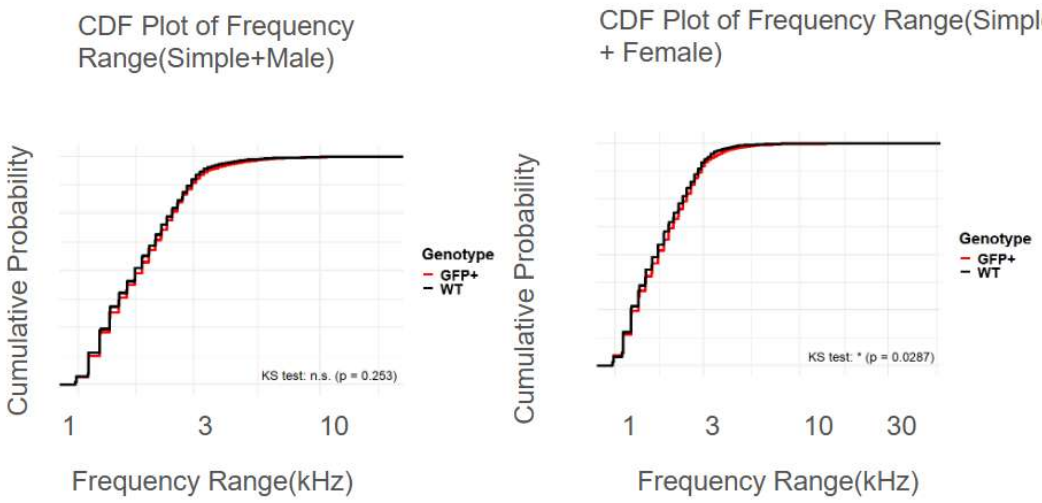

Figure 2: Cumulative distribution plots of vocalization frequency range (log scale) for wild-type (WT) and mutant mice. The left panel shows simple male vocalizations, and the right panel shows simple female vocalizations.

3.3. Maximum Acoustic Power

Thirdly, we analyzed whether the maximum energy level (maximum acoustic power) of vocalizations differs between wild-type and mutant mice. When examining simple vocalizations, male mutant mice did not differ significantly from male controls (Figure 3, left panel; KS test, p = 0.379). In contrast, female mutant mice exhibited significantly higher maximum acoustic power compared to female controls (Figure 3, right panel; KS test, p = 0.017). These results suggest that the A53T mutation influences vocal intensity primarily in simple vocalizations, with a more pronounced effect in females, while males remain unaffected.

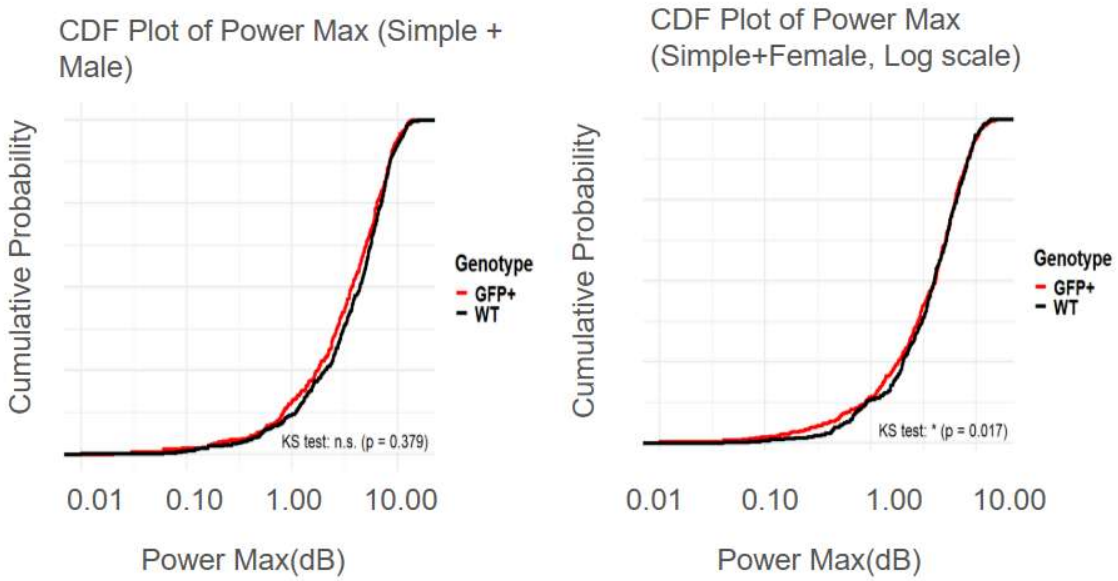

Figure 3: Cumulative distribution plots of maximum acoustic power (log scale) for wild-type (WT) and mutant mice. The left panel shows simple male vocalizations, and the right panel shows simple female vocalizations.

3.4. Summary of Results

Overall, the analyses revealed that simple vocalizations were differentially affected by the A53T mutation in male and female mice. For vocalization duration, no significant differences were observed between mutant and wild-type animals in either sex. In contrast, frequency range showed a selective genotype effect: female mutant mice exhibited significantly broader frequency ranges compared to their controls, while males showed no measurable difference. For maximum acoustic power, differences were also sex-specific, with female mutants showing considerably higher values than female controls, while no difference was found in males. Together, these findings suggest that frequency-related measures are the most sensitive markers of genotype differences in females, while vocal intensity changes are likewise more pronounced in females.

4. Discussion

Summary of Key Findings

4.1. Vocalization Duration

Simple vocalizations were examined to assess whether the A53T mutation altered call timing across sexes. Mutant mice did not differ significantly from wild-type controls in the duration of simple calls for either males or females (Figure 1). This indicates that call length is not measurably influenced by the mutation, suggesting that duration is a relatively stable

parameter across genotypes and sexes.

4.2. Frequency Range

Analysis of simple calls revealed a female-specific effect of the A53T mutation. Mutant females exhibited significantly broader frequency ranges compared to WT females, whereas no measurable differences were observed in males. This indicates that sex may modulate the acoustic breadth of simple calls, with frequency range serving as a sensitive marker of genotype-related differences in females.

4.3. Acoustic Power

Maximum acoustic power showed a sex-specific effect of the A53T mutation in simple vocalizations. Female mutants exhibited significantly higher values than WT females, whereas no measurable differences were observed in males. These findings suggest that simple calls are sensitive to mutation-driven changes in vocal intensity, with effects that are more pronounced in females.

4.4. Comparisons with Other Studies

A previous study using Thy1-aSyn rats reported a reduction in the duration, frequency, and intensity of ultrasonic vocalizations, with these abnormalities worsening over time (Grant et al., 2014). In contrast, our analysis of A53T mice revealed a significantly broader frequency range in females, as well as higher maximum power in females, while duration showed no significant differences. These discrepancies likely reflect methodological variations such as species, age, classification criteria, or acoustic analysis approaches. Importantly, unlike the original study, which emphasized overall impairments, our analysis uncovered female-specific alterations, highlighting that α-synuclein pathology may differentially affect vocal motor circuits across sexes.

Similarly, Gombash et al. (2013) found reduced vocal intensity in rats, but no consistent changes in frequency or duration. In contrast, our analysis of A53T mice revealed a broader frequency range and higher maximum power in females, while duration showed no significant differences. These findings suggest that, although reduced intensity is a common outcome of α-synuclein pathology, additional alterations in frequency may emerge in a female-specific manner.

Paumier et al. (2015) reported that α-synuclein pre-formed fibril (PFF)-injected rats exhibited shorter call durations and reduced vocal intensity. In contrast, our analysis of A53T mice revealed no significant differences in duration but did find a broader frequency range and higher maximum power in females. These differences may be explained by model-specific factors, since PFF injections produce progressive pathology, whereas A53T transgenics exhibit constitutive α-synuclein overexpression.

Together, these findings suggest that although α-synuclein pathology consistently affects vocal intensity, its impact on duration and frequency range may depend on the disease model and sex.

4.5. Implications for Neural Mechanisms

The alterations we observed in simple vocalizations suggest that α-synuclein pathology disrupts neural circuits critical for

basic vocal motor control. Simple ultrasonic calls are thought to be mediated by brainstem and basal ganglia pathways, with dopaminergic modulation playing a key role in shaping frequency and intensity (Ciucci et al., 2007; Tschida et al., 2019). The broader frequency ranges in females and increased vocal intensity in females may reflect dysfunction in basal ganglia–brainstem circuits, alongside sex-dependent modulation of dopaminergic pathways. These findings highlight the basal ganglia as a central locus of vulnerability in PD-related vocal deficits and suggest that sex-specific factors, such as hormonal influences, may amplify circuit dysfunction.

Sex differences in dopaminergic function and hormonal modulation may underlie the female-specific effects observed. Estrogen and related factors influence dopamine signaling within basal ganglia circuits, and disruption of these interactions by α-synuclein overexpression could exaggerate alterations in vocal frequency and intensity in females. Human studies report similar patterns: although women generally show a lower incidence and slower motor progression of Parkinson's disease, they often present with distinct non-motor symptoms, including differences in speech prosody and vocal control (Baldereschi et al., 2000; Haaxma et al., 2007; Solla et al., 2012; Abraham et al., 2019; Picillo et al., 2022; Santos-García et al., 2023). These parallels suggest that sex hormones and dopaminergic tone jointly modulate the vulnerability of vocal circuits, offering a biological explanation for the female-specific alterations identified in A53T mice.

These findings underscore the scientific relevance of vocalization analysis in PD models, as changes in call structure may serve as behavioral readouts of underlying neural dysfunction. Because vocal production depends on the precise motor control of respiration and laryngeal muscles, as well as cognitive sequencing of call patterns, disruptions in vocalizations can reflect both motor and cognitive impairments characteristic of PD (Rektorová et al., 2012; Grant et al., 2014; Ciucci et al., 2007). This highlights the potential of vocalization metrics as non-invasive biomarkers for early detection and monitoring of disease progression (Bocklet et al., 2011; Grant et al., 2015).

4.6. Translational Relevance of Behavioral and Pharmacological Approaches

Our findings reveal female-specific alterations in frequency range and acoustic power in A53T mice, suggesting that α-synuclein pathology interacts with sex-dependent dopaminergic modulation of vocal control circuits. These results form the basis of a translational framework that links preclinical vocal biomarkers to clinical interventions targeting the same neural pathways. The increased vocal power observed in female A53T mice may reflect compensatory activation within dopaminergic circuits of the basal ganglia and brainstem—pathways that are similarly targeted by behavioral therapies in humans. This correspondence between preclinical and clinical patterns supports the use of vocalization metrics as a bridge for evaluating circuit-level effects of treatment intervention.

Human studies have demonstrated the effectiveness of the Lee Silverman Voice Treatment (LSVT) in improving vocal deficits in Parkinson's disease. LSVT enhances vocal loudness, articulation, and clarity through high-effort vocal exercises, with improvements reported in vocal intensity, articulation, and perceptual speech quality (Ramig et al., 2001; Silveira & Brasolotto, 2005; Searl et al., 2011; Cannito et al., 2012; Sapir et al., 2007). Pharmacological treatments such as levodopa, in contrast, have shown mixed effects on vocal outcomes. Some studies have reported limited improvements in vocal quality (De Letter et al., 2006; Kelm-Nelson et al., 2016), while others have observed increased loudness and speech speed, but without sustained benefits (Ho et al., 2008). These findings suggest that combining pharmacological treatments with behavioral therapies, such as LSVT, may offer stronger outcomes than medication alone.

Testing such strategies in animal models could therefore clarify underlying mechanisms and therapeutic potential. By connecting the variability observed in human interventions with the patterns identified in our animal vocalization data, we provide a translational framework that highlights how preclinical studies can inform combined treatment approaches for Parkinson's vocal deficits.

4.7. Limitations of This Study

Two limitations should be noted. First, the cross-sectional design prevents conclusions about the progression of vocal impairments over time. Second, the hormonal cycle in females was not controlled, which may influence vocalization parameters such as frequency or intensity (Geyer & Barfield, 1978). Some observed differences could therefore be partially attributable to hormone-related variability.

4.8. Future Research Directions

Future work should employ longitudinal designs to track vocal symptoms over time and correlate them with neurodegenerative changes. Such studies could identify early markers of disease progression and clarify how vocal deficits develop over time with age.

Additionally, targeted neurobiological investigations are needed to map the specific brain regions controlling simple vocalizations and to determine how A53T alters these circuits. Integrating vocal-behavior studies with therapeutic interventions, including both behavioral and pharmacological approaches, could accelerate the development of non-invasive biomarkers and treatment strategies for Parkinson's disease.

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Mentor Contribution Statement

Daniel Kim served as the academic mentor supervising this research project. He provided guidance throughout the development of the manuscript, especially in refining the research framework and ensuring methodological accuracy. During the writing process, he advised on data interpretation and the structure of the discussion section, encouraging logical consistency and depth of analysis. His mentorship focused on cultivating independent critical thinking rather than prescribing conclusions, allowing the author to take full ownership of the research direction. While the core research, analysis, and writing were conducted independently by the student, his feedback was instrumental in improving the overall quality and rigor of the final paper. I believe I have submitted all the required things, so please let me know if there is any further information I can provide.

Appendix

Table 1: Comparison of vocalization duration (p-values) between wild-type (WT) and mutant mice. Results are shown separately for simple and complex calls, and further divided by sex.

Supplementary Table 1. Duration comparisons (p-values)

Condition	WT vs mutant	Male WT vs mutant	Female WT vs mutant
Duration / Simple	0.02	0.18	0.18
Duration / Complex	0.04	0.09	0.13

Table 2: Frequency comparisons (p-values) between wild-type (WT) and mutant mice. Results are shown separately for simple and complex vocalizations, and further divided by sex.

Supplementary Table 2. Frequency comparisons (p-values)

Condition	WT vs mutant	Male WT vs mutant	Female WT vs mutant
Frequency / Simple	0.01	0.25	0.03
Frequency / Complex	0.35	0.58	0.40

Table 3: Power comparisons (p-values) between wild-type (WT) and mutant mice. Results are shown separately for simple and complex vocalizations, and further divided by sex.

Supplementary Table 3. Power_max comparisons (p-values)

Condition	WT vs mutant	Male WT vs mutant	Female WT vs mutant
Power_max / Simple	0.03	0.38	0.02
Power_max / Complex	0.21	0.23	0.21