Decoding sex-specific vocal repertoire and syntactic usage in the Fragile X mouse model of autism

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Abstract

Pup-dam ultrasonic vocalizations (USVs) contribute to the formation of neural circuits and behaviors essential for standard cognitive and socio-emotional development. In conditions like autism and Fragile X Syndrome (FXS), disruptions in pup-dam USV communication hint at a possible connection between abnormal early developmental USV communication and the later emergence of communication and social deficits. Syntax, a crucial element of rodent "language," has rarely been investigated in FXS mice, let alone in specimens of both sexes. Therefore, in this study, we gathered USVs from PND 10 FXS pups during a short period of separation from their mothers, encompassing animals of all possible genotypes and both sexes (i.e., *Fmr1-/y*vs. *Fmr1+/y* males and *Fmr1+/+*, *+/-*, and *-/-*females). This allowed us to compare, for the first time, the relative influence of sex and gene dosage on their communication capabilities. Leveraging DeepSqueak and analyzing vocal patterns, we examined intricate vocal behaviors such as call structure, duration, frequency modulation, and temporal patterns. The results demonstrate that FMRP-deficient pups of both sexes display an increased inclination to vocalize when separated from their mothers, and this behavior is accompanied by significant sex-specific changes in the main features of their USVs as well as in body weight. Moreover, the decoding of the vocal repertoire and its syntactic usage revealed that the silencing of the *Fmr1* gene primarily alters the qualitative composition of ultrasonic communication in males. These findings highlight the fascinating interplay between *Fmr1* gene dosage and sex in shaping communication during infancy.

Introduction

Ultrasonic vocalizations (USVs), serving as a vital pathway for examining the complexities of mouse communication within their social behavioral context[1], have garnered escalating prominence in the fields of psychiatry and neurology. The relevance is notably profound in the context of conditions marked by compromised social interaction and communication, such as neurodevelopmental disorders (NDDs) and autism spectrum disorders (ASDs), as evidenced by numerous studies[1–6]. This accentuates the compelling capacity of USV studies to enhance our comprehension of animal behavior, particularly regarding disorders that impede regular social interaction. The developmental trajectory of USVs, particularly within the 30 to 110 kHz range[7], remains consistent across majority of mouse strains. Hence, the frequency of USV calls usually escalates during the initial 5-6 days following birth, reaching a peak around the sixth or seventh postnatal day (PND). Following this peak, call rates begin to diminish and typically cease by the end of the second postnatal week. Notably, the precise timing of these transitions could be strain-dependent, with C57BL/6 mice, for instance, typically displaying the highest USV rate around PND 3[8,9]. To scrutinize this form of communication, researchers commonly employ three experimental protocols: isolation-induced USVs in pups, interaction-induced USVs in both young and adult mice, and courtship-induced USVs in adult mice[5].

First described as "whistles of loneliness" due to their role in provoking maternal care and fostering communication between mother and offspring[10], USVs are a vital communication mechanism for mouse

pups in their early weeks of life, and their frequency tends to increase when the pups are isolated from their nest, mother, and siblings[5]. Multiple studies have examined communication abilities in mouse models of Fragile X Syndrome (FXS), yielding diverse results depending on the strain, protocol, and age of the mice[11–19].

Given its prevalence (1.4 per 10,000 males and 0.9 per 10,000 females in the total population[20]), FXS is the foremost inherited cause of intellectual disability (ID) and the most common syndrome linked with ASD[20,21].

FXS patients often present a broad spectrum of physical, neurological, social, behavioral, and cognitive anomalies[22]. A salient feature of FXS involves deficits in communication, where delays in speech and language development are commonplace. This atypical trajectory of language development is strongly intertwined with intellectual capabilities and autism traits[23]. Children diagnosed with FXS frequently exhibit speech patterns marked by compulsion, repetition, and perseverance[24]. Expressive language delays are a common observation in both male and female FXS patients, with the severity of effects typically being less in females owing to the X-linked nature of the disorder[25,26].

Here we exploited the transformative capacity of deep learning and implemented DeepSqueak[27] to evaluate USVs across all genotypes and sexes in the widely used mouse model for FXS (*Fmr1*-KO2[28]). Our findings revealed that the considerable quantitative and qualitative impact of fragile x messenger ribonucleoprotein 1 (FMRP) on vocalization syntax is highly dependent on both sex and gene dosage, thereby influencing the structure of USVs. The study asserts the vital role of FMRP as a regulator of communicative behaviors and underlines the imperative of considering sex-based differences in understanding its influence.

Results

Fmr1 silencing induces sex-dependent alterations in body weight.

Our first order of investigation involved analyzing the body weights of the mice involved in the study. We found that the absence of the *Fmr1* gene leads to sex-specific changes in body weight. Interestingly, we noticed a discernible difference in weight between the male and female mice at PND 10 when FMRP was absent. Specifically, we discovered that in the FMRP-absent scenario, male mice weighed less, while female mice were heavier as compared to their respective control groups (**Fig. 1**; **Suppl. Tab. 1**). Furthermore, when considering the control animals (i.e., where FMRP was not manipulated), we observed that female mice typically weighed less than their male counterparts (as shown in **Fig. 1**; **Suppl. Tab. 1**). These observations underscore the role FMRP plays in body weight regulation and the inherent sex-dependent differences that exist, further emphasizing the complexity of the molecular and physiological mechanisms at play.

Heightened vocalization responses in FMRP-deficient pups during maternal separation.

Upon separation from their mothers, pups lacking FMRP in both sexes demonstrated a higher frequency of vocalizations compared to those in the control group. Notably, there was no such change in the partially deficient (*+/-*) females (refer to **Fig. 2A**; **Suppl. Tab. 2**). Furthermore, complete absence of FMRP led to quicker vocal response in females, whereas in males, the response time remained comparable to the control group (**Fig. 2B**; **Suppl. Tab. 2**). When examining the proportion of vocalizing (V) and non-vocalizing (NV) pups across the groups, it was found that both male and female pups deficient in FMRP (*-/y* and *-/-* respectively) had a higher percentage of vocalizers compared to their respective control groups (*+/y* and *+/+* respectively) (refer to **Fig. 2C**; **Suppl. Tab. 2**). Overall, this simple analysis indicates that the lack of FMRP enhances the likelihood of vocalizing in both sexes during maternal separation.

Sex-specific impact of FMRP deficiency on fundamental USV Characteristics.

We then investigated the four fundamental characteristics of USVs: length, primary frequency, power, and frequency range. The data showed that female control mice produced longer USVs compared to their male counterparts (**Fig. 3A**; **Suppl. Fig. 1A**; **Suppl. Tab. 3**). Notably, when FMRP was absent, male mice (*-/y*) generated longer USVs than their normal counterparts (*+/y*). This effect, however, was male-specific, as FMRP deficiency did not yield longer USVs in female mice, regardless of being partially or totally deficient (**Fig. 3A**; **Suppl. Tab. 3**). Frequency distribution analysis supported these observations, indicating increased use of longer USVs in FMRP-deficient male mice compared to controls (**Fig. 3B**). Conversely, female mice displayed a contrasting trend, with control (*+/+*) females using longer USVs more frequently than partially or fully deficient females (**Fig. 3C**), despite similar average lengths across these female groups (**Fig. 3A**; **Suppl. Tab. 3**). Analysis of the primary frequency of USVs across all groups revealed no significant variations. Although average frequencies were comparable (**Fig. 3D**; **Suppl. Tab. 3**), control females tended to use lower frequencies more frequently than males (**Suppl. Fig. 1C**). This gender difference was less pronounced in FMRP-deficient mice (**Suppl. Fig. 1D**). Comparing the mean power of USVs, males showed little change regardless of FMRP status, whereas FMRP-deficient females shifted towards more negative powers (**Fig. 3G**; **Suppl. Tab. 3**). Further analysis indicated that both FMRP-deficient males and females used USVs with more negative power more often than their respective controls (**Fig. 3H, I**). Lastly, the average frequency range used in vocalizations did not show any notable variations based on sex or genotype (**Fig. 3J**; **Suppl. Tab. 3**). Nonetheless, frequency distribution analysis suggested a wider range in FMRP-deficient males than in controls (**Fig. 3K**), with no such difference in females (**Fig. 3L**). Typically, females use larger frequency ranges more often than males (**Suppl. Fig. 1G**), but this difference disappeared in the absence of FMRP (**Suppl. Fig. 1H**). In conclusion, FMRP deficiency not only impacted the frequency of vocalizations, but also led to sex-specific alterations in the properties of ultrasonic communication.

Absence of FMRP spurs differences in vocal repertoire.

Leveraging the advanced call classification capabilities of DeepSqueak[27], we pinpointed 10 unique types of USVs within our dataset, as depicted in **Fig. 4A**. Close examination of each group vocal repertoire uncovered a fascinating hierarchy in the use of these different USVs during maternal separation (**Fig. 4B,**

D; **Suppl. Tab. 4,5**). Through a statistical comparison of these vocal profiles across various genotypes (**Fig. 4C, E**; **Suppl. Tab. 6**), we found a striking difference. In male mice lacking FMRP, there was a substantial decrease in the use of 'Short' vocalizations (**Fig. 4C**; **Suppl. Tab. 6**). In contrast, female vocal repertoire remained unaltered in the absence of FMRP (**Fig. 4E**; **Suppl. Tab. 6**). When analyzing vocalizations based on sex, male and female control mice showed similar vocal profiles. However, in FMRP-deficient mice, the picture changed: males used 'Inverted-U' vocalizations less often and 'Flat' vocalizations more frequently compared to their female counterparts (**Suppl. Tab. 6**). Despite the absence of FMRP having a significant effect, all groups still utilized the same ten vocalizations. Most notably, the lack of FMRP predominantly affected the vocal repertoire of male mice.

Decoding vocal transitions: FMRP deficiency differentially shapes the syntax of male and female mice.

Finally, we turned our attention to analyzing the likelihood of transitioning between different types of vocalizations within the syntax patterns of our various test groups (**Fig. 5,6**; **Suppl. Fig. 2; Suppl. Tab. 7-12**). By scrutinizing the transition probability from and to each type of USV within each group, we discovered unique patterns of vocalization use in their syntax (**Fig. 5A-D**, **6A-F**; **Suppl. Tab. 7,8,10,11**). Statistical comparison between genotypes revealed that FMRP deficiency alters the likelihood of transitioning from 'Downward Ramp', 'Complex Trill', and 'Short' USVs in male pups (**Fig. 5E**; **Suppl. Tab. 9**). Intriguingly, the probability of transitioning to different types of USVs remained unchanged between FMRP-normal and deficient males (**Fig. 5F**; **Suppl. Tab. 9**). Conversely, in female pups, FMRP deficiency did not seem to significantly impact the transition probabilities from and to various USVs (**Fig. 6G, H**; **Suppl. Tab. 9**). In a nutshell, qualitative arrow diagram and heat map analysis unveils the complex fabric of communication, spotlighting a more profound influence of FMRP on the syntax of male mice (**Fig. 5G; Suppl. Fig. 2A**) as opposed to females (**Fig. 6I; Suppl. Fig. 2B**).

Discussion

This study sheds light on the complex relationship between *Fmr1* gene dosage, sex, and communication development during infancy. Through analyzing vocal patterns and intricate vocal behaviors, we observed that FMRP-deficient pups of both sexes exhibited an increased tendency to vocalize when separated from their mothers. However, these vocalizations were accompanied by significant sex-specific changes in the main features of their USVs and impacted body weight. We found that the silencing of the *Fmr1* gene primarily affected the qualitative composition of ultrasonic communication in males.

Importantly, this study is the first to investigate the influence of FMRP on communication in both sexes using this specific genetic model during infancy. Notably, we generated and analyzed pups with various genotypes, including *+/y* and *-/y* males, as well as *+/+*, *+/-*, and *-/-* females. The *-/-* condition is rare in human females[30], and until now, there has been a lack of rodent data on this subject.

During the early stages of NDDs, variations in metabolism and body weight are commonly observed[31]. In individuals with FXS, these alterations lead often to obesity as they age[32]. Previous studies in FXS mouse

models have reported higher body weight in adult males (*-/y*) and females (*-/-*) compared to their respective controls[31], but there is limited research on infant body weight using the same FXS model. Interestingly, our study found that male *-/y* mice had lower weight than *+/y* mice, while in females, *-/-* mice had higher weight than *+/+*. This suggests a sex-specific role of FMRP in metabolism during childhood, and one can hypothesize that the lower body weight in males without FMRP is due to impaired maternal care resulting from communication deficits. Further investigations are needed to understand this phenomenon and its implications within the complex framework of sex-specific nuances in FXS pathology.

During early life, USVs serve as the primary mode of communication in rodents, providing a valuable window to gain insights into the initial stages of NDDs and ASDs[1–6]. Maternal separation is a widely utilized technique to evoke meaningful USVs in rodents. In our study, we observed that maternal separation resulted in higher USV emission in both male and female FMRP-deficient mice. Interestingly, only in females, we noticed a decrease in the latency for the first vocalization. This heightened vocalization propensity in FXS mice may indicate a distinct emotional response to separation compared to control mice[1,5]. Previous research has demonstrated an increased anxious phenotype in FXS male mice during this developmental period[17], which aligns with an augmented call for maternal care upon nest removal. Moreover, it has been observed that children with FXS often exhibit compulsive, repetitive, and perseverative speech[24]. Thus, it is plausible to speculate that the increased vocalization propensity observed in FMRP-deficient mice might reflect a similar aspect of the pathology.

Although the absence of FMRP resulted in an increased vocalization rate in both males and females, the specific characteristics of vocalizations exhibited sex-specific differences. Notably, only males showed a longer average length of USVs in the absence of FMRP, while females did not exhibit this change. Interestingly, another study in a different strain also reported sex-specific alterations in vocalization length, indicating a generalization of this phenotypic trait[18]. Consistent with this notion, a similar pattern of changes in both the quantity and quality of pup communication has been observed in the NF-κB p50-KO mice model of NDD. These mice emit a higher number of USVs and longer USVs when separated from their mothers, compared to control mice[33]. This shared characteristic across NDD models suggests that the amount and duration of USVs may reflect important aspects related to developmental status or cognitive abilities in mice. Further investigations are warranted to delve into the underlying implications of these alterations.

Through extensive investigation into the probability of transitioning between different types of USVs, we have uncovered that the absence of FMRP leads to sex-specific variations in vocal syntax of FXS pups. Our current observation aligns with observations made in the development of oral communication in FXS children that display limited expressive syntax[34–38]. Specifically, we observed significant differences in the usage of 'Downward Ramp', 'Complex Trill', and 'Short' calls within the syntax of male *-/y* compared *+/y* controls. In contrast, females exhibited a high degree of similarity in the syntactic usage of the ten different types of USVs across various genotypes. This finding, along with the aforementioned

alterations, provides evidence of the sex-specific impact caused by the absence of FMRP on communication quality during early development. Thus, males are considerably more impacted than females. In human patients too, alterations are more pronounced in males than females[25] that do not display complexity deficits[38]. When assessing the expressive language capabilities of male and female patients with FXS, females typically perform better. The considerable variation between individuals, could be partly attributed to differences in the activation ratios of the X chromosome[37,39].

These parallel findings emphasize the importance of considering sex differences in understanding the effects of FMRP deficiency on communication, highlighting the need for sex-specific approaches in the study of NDDs.

The impact of FXS on communication in females is typically milder due to the presence of one unmutated gene copy[25,37–39]. To further investigate the effects of complete FMRP absence on both sexes, we conducted a study involving homozygous (-/-) females. Interestingly, the findings revealed that certain communication features, such as vocalization propensity and power of USVs, were abnormal in homozygous females but not in heterozygous females, suggesting that a single unmutated gene copy is sufficient to maintain normal functions in females. Moreover, the vocal repertoire and syntax of homozygous females were preserved compared to those of males with FMRP deficiency.

In conclusion, these findings illuminate the complex interplay between *Fmr1* gene dosage, sex, and communication growth during infancy, along with the intricacies of Fragile X Syndrome pathology. In addition, they emphasize the paramount importance of considering sex differences when researching neurodevelopmental disorders.

Methods

Animal

Animals were treated in compliance with the European Communities Council Directive (86/609/EEC) and the United States National Institutes of Health Guide for the care and use of laboratory animals. *Fmr1*-KO2 mice from FRAXA foundation were used in this study. Females *Fmr1+/-* were paired with males *Fmr1+/y* or *-/y* to obtain all genotypes included in this study (males *+/y*, males *-/y*, females *+/+*, females *+/-* and females *-/-*). The male was removed from the cage after 1 week from the beginning of the mating. The behavioral tests were performed in male and female offspring during PND 10. All mice used in this study were housed in standard wire-topped Plexiglas cages (42 × 27 x 14 cm) in a temperature and humidity-controlled condition (i.e., temperature 21 ± 1 °C, 60 ± 10% relative humidity and 12 h light/dark cycles). The nesting material was standardized providing 15 grams of aspen pad and 1 compressed cotton stick. Food and water were available ad *libitum*.

Ultrasound vocalizations

USVs were elicited through a rapid maternal separation procedure conducted on male and female pups at PND 10[29]. Each mouse was individually placed in an empty plastic container measuring 11 x 7 x 3.5 cm, which was located inside a sound-attenuating isolation box. USVs were captured using an ultrasonic microphone (Ultravox Noldus), connected to the Ultravox device (Noldus, Netherlands), and positioned 20 cm above the pup within its plastic container. Following the 4-minute recording session, each pup was weighed, had its body temperature measured, and a sample of tail tissue was collected for genotype determination.

The acoustic traces in the individual audio files were identified and studied using DeepSqueak[27] (version 2.6.2). This software converted the files into corresponding sonograms and utilized a Faster-RCNN object detector for analysis. To focus on the pertinent frequency range and reduce the interference of unrelated noise, a frequency band spanning from 20 kHz to 100 kHz was set as the minimum and maximum cutoff frequencies, respectively. Each sonogram was then transformed into the corresponding spectrogram, and any call identified as noise was manually excluded. Automated USVs classification, pattern analysis and transition probabilities computation were performed in DeepSqueak through a neural network specifically designed for mouse call classification. This enabled the examination of ten distinct vocalization types: Complex, Downward ramp, Inverted-U, Upward ramp, Complex trill, Short, Step Down, Flat, Step up, and Trill.

Statistical analysis

In the initial stage, each animal was evaluated to determine their body weight and propensity to vocalize. This involved tallying the number of USVs, while also studying the latency and percentage of vocalizers. Following this, a more in-depth analysis was performed on animals demonstrating a baseline level of vocalizations, thus ensuring the software had ample data for detecting multiple transitions between USVs (>1). This investigation included studying a range of characteristics of the USVs, such as their duration, primary frequency, power, and change in frequency. Additionally, the vocal repertoire and syntax were analyzed to understand overarching patterns and structure in the animal vocalizations.

The datasets were assessed for normality using the D'Agostino-Pearson and Shapiro-Wilk tests. Given that none of the datasets met the prerequisites for parametric analyses, including normality and uniform sample sizes, the Mann–Whitney U test was employed for conducting statistical comparisons. GraphPad Prism 9 and DeepSqueak 2.6.2 were utilized for performing the statistics. The N values correspond to the number of animals tested in each group. Statistical significance was established at $p < 0.05$, with exact *p*-values indicated in the figures and tables.

Declarations

Ethics approval

Animals were treated in compliance with the European Communities Council Directive (86/609/EEC) and the United States National Institutes of Health Guide for the care and use of laboratory animals.

Availability of data and materials

All data reported in this paper will be shared by the lead contact upon request. This paper does not report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Competing interest

The authors declare no competing interests.

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Authors' contributions

GG: Conceptualization, Data curation, Formal analysis, Validation, Writing—original draft, review and editing. DI: Data curation. AC-R: Data curation. BS: Formal analysis. PC: Conceptualization, Supervision, Methodology, Writing—, review and editing. OJM: Conceptualization, Supervision, Funding acquisition, Methodology, Project administration, Writing—original draft, review, and editing.

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Figures

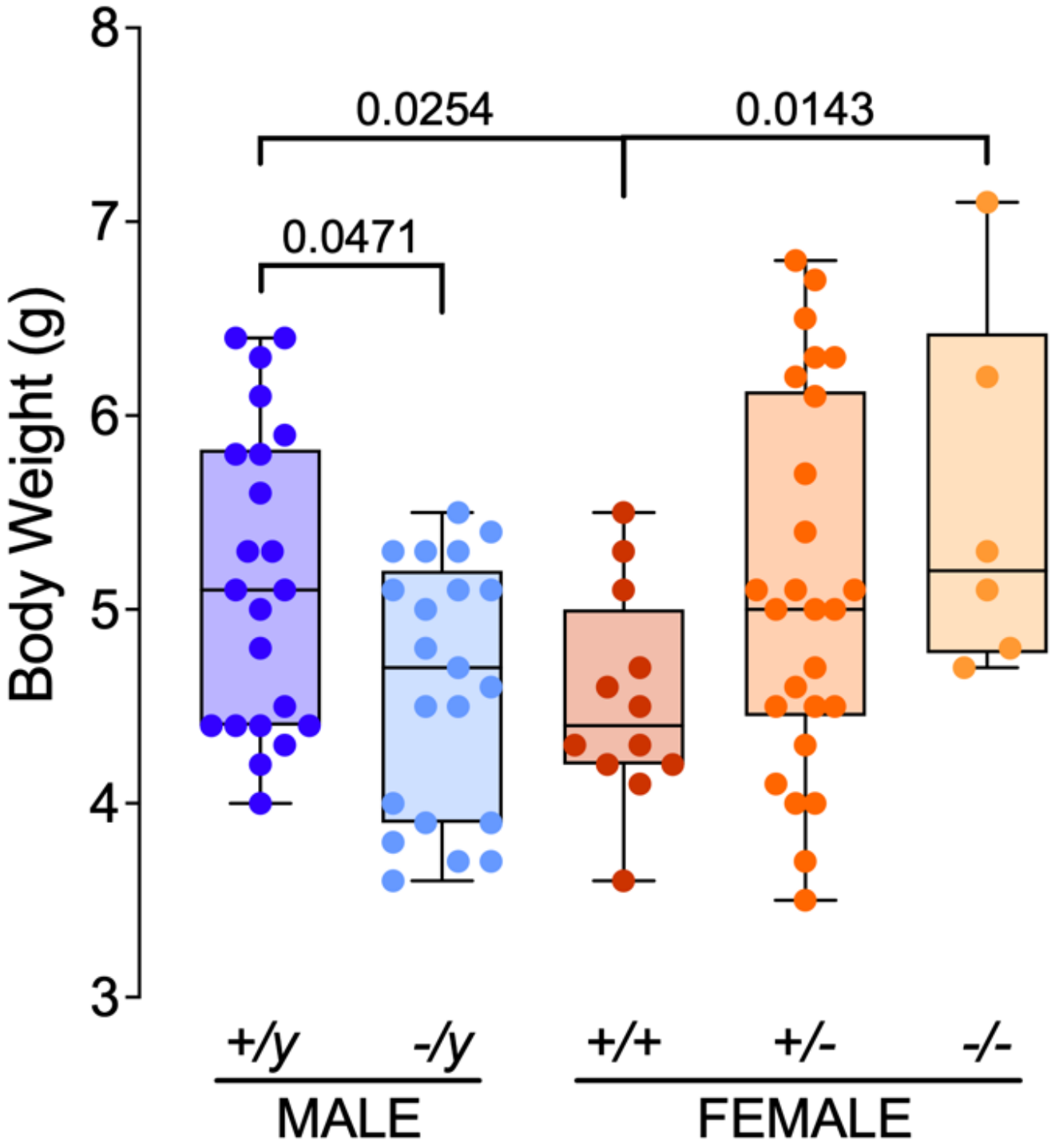

Figure 1

FMRPP and sex influence body weight.

When FMRP is absent, the body weight of male mice (*-/y*) decreases, whereas female mice (*-/-*) show an increase in weight compared to their respective control groups. Among the control groups, male mice (*+/y*) are typically heavier than female mice (*+/+*). Each data point in the plot signifies an individual mouse. The box plots present the data ranging from minimum to maximum values, with median and interquartile range (25-75 percentile) shown. The Mann-Whitney U test was applied for statistical analysis. *P*-values less than 0.05, indicating statistical significance, are marked on the graphs, while complete statistics can be found in Table 1. Sample size: *+/y* males N=22, *-/y* males N=21, *+/+* females N=12, *+/-* females N=26 and *-/-* females N=6.

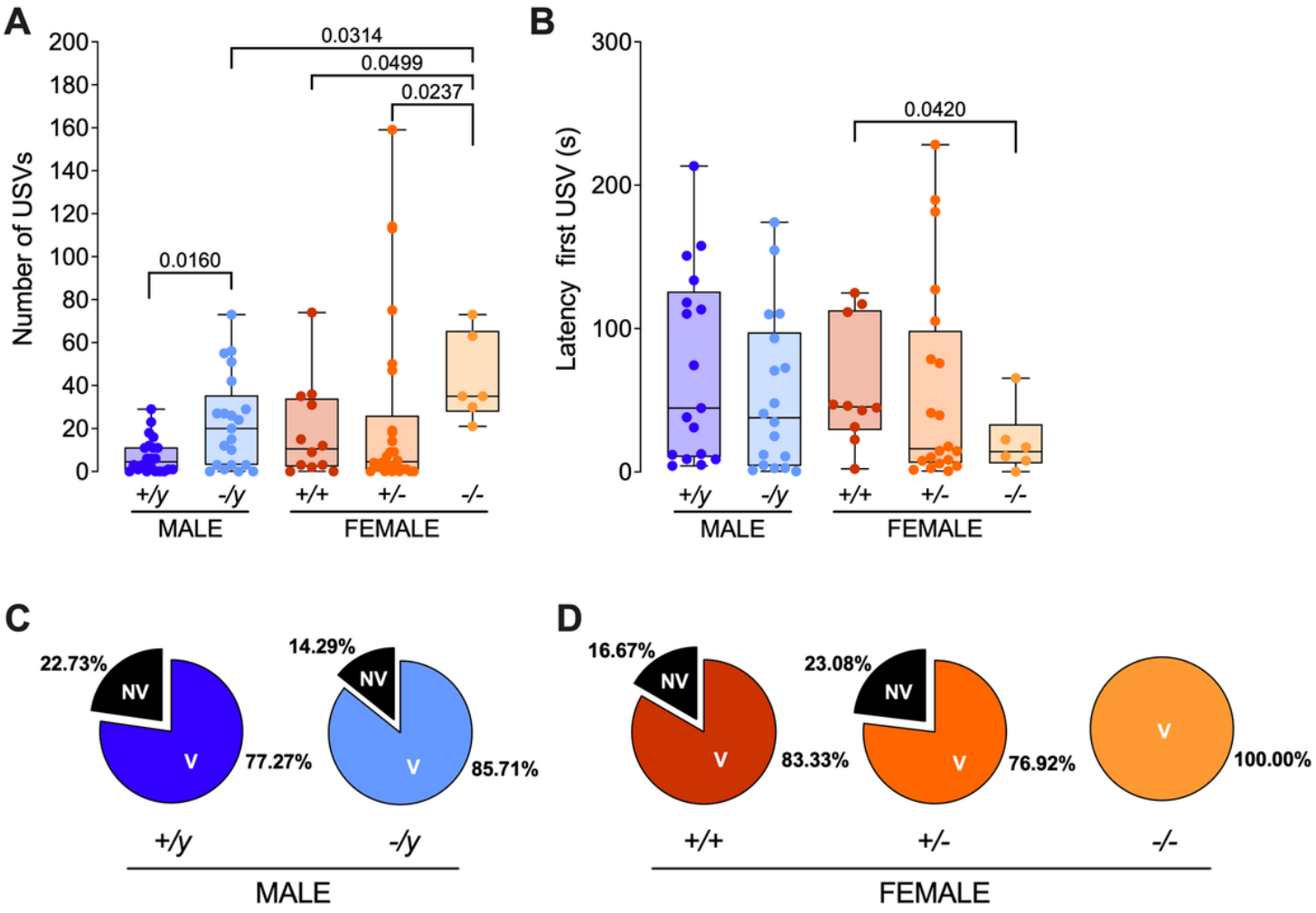

Figure 2

Sex-specific differences in vocalizations and vocalization latency in FXS Pups.

(**A**) FXS pups of both sexes display a higher number of vocalizations compared to their control counterparts. (**B**) Only females show a shorter vocalization latency in the absence of FMRP. (**A, B**) Data are presented as min. to max. box plots with median and 25-75 percentile, where each dot represents an

individual mouse. Mann-Whitney U tests were conducted, and *p*-values <0.05 are indicated in the graphs. Full statistical details can be found in Table 2. (**C, D**) Pie graphs illustrate the percentages of vocalizing (V) and non-vocalizing (NV) male (**C**) and female (**D**) pups. The percentages were calculated by dividing the number of vocalizers or non-vocalizers by the total number of animals tested in each group. Sample sizes : (**A, C, D**) *+/y* males N=22, *-/y* males N=21, *+/+*females N=12, *+/-* females N=26, *-/-* females N=6. (**B**) *+/y*males N=17, *-/y* males N=18, *+/+* females N=10, *+/-* females N=20 and *-/-* females N=6.

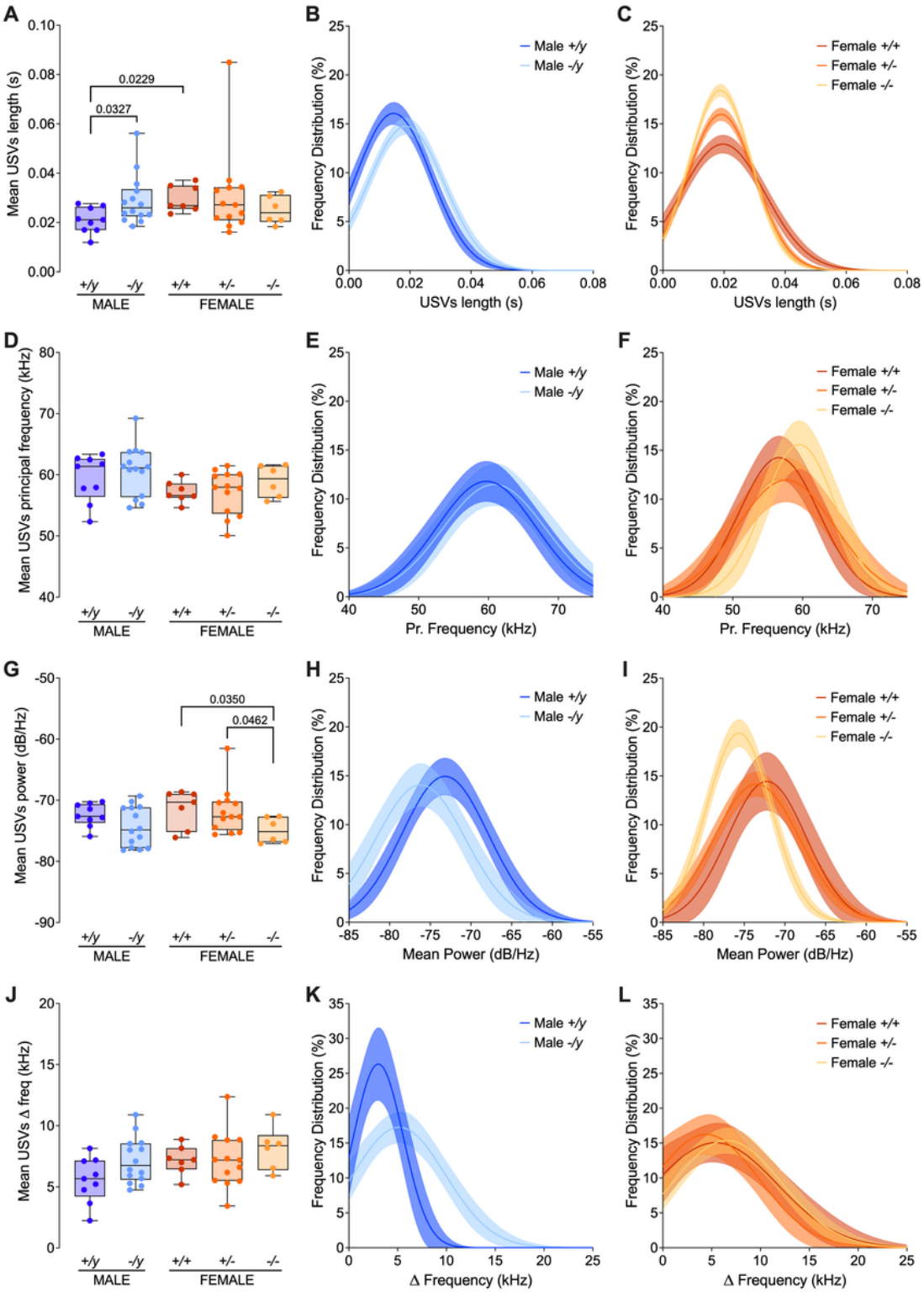

Figure 3

Sex-dependent alteration of core features in USVs of FMRP-deficient mice.

(**A**) FMRP deficiency specifically leads to longer mean length of vocalizations in males. (**B, C**) Frequency distribution (%) of USV length shows opposite impacts in male (**B**) and female (**C**) pups. (**D-F**) The principal frequency of vocalizations remains similar across the groups. (**G**) In the absence of FMRP, only females exhibit a statistically more negative mean power in their USVs. (**H, I**) Frequency distribution analysis reveals that both sexes show a greater utilization of USVs with more negative power in FMRP-deficient pups. (**J-L**) The mean change in frequency of USVs does not appear to be affected by the FXS genotype in either sex (**J**), but frequency distribution analysis indicates wider delta use in the absence of FMRP in males (**K**) but not in females (**L**). (**A, D, G, J**) Single dots represent individual mice, and the data are presented as min. to max. box plots with median and 25-75 percentile. Mann-Whitney U tests were performed, and *p*-values <0.05 are indicated in the graphs. Full statistical details can be found in Table 3. (**B, C, E, F, H, I, K, L**) Data are represented as a Gaussian curve fit (±CI) of the frequency distribution (%). Sample sizes: (**A–L**) *+/y* males N=9, *-/y* males N=14, *+/+*females N=7, *+/-* females N=13 and *-/-* females N=6.

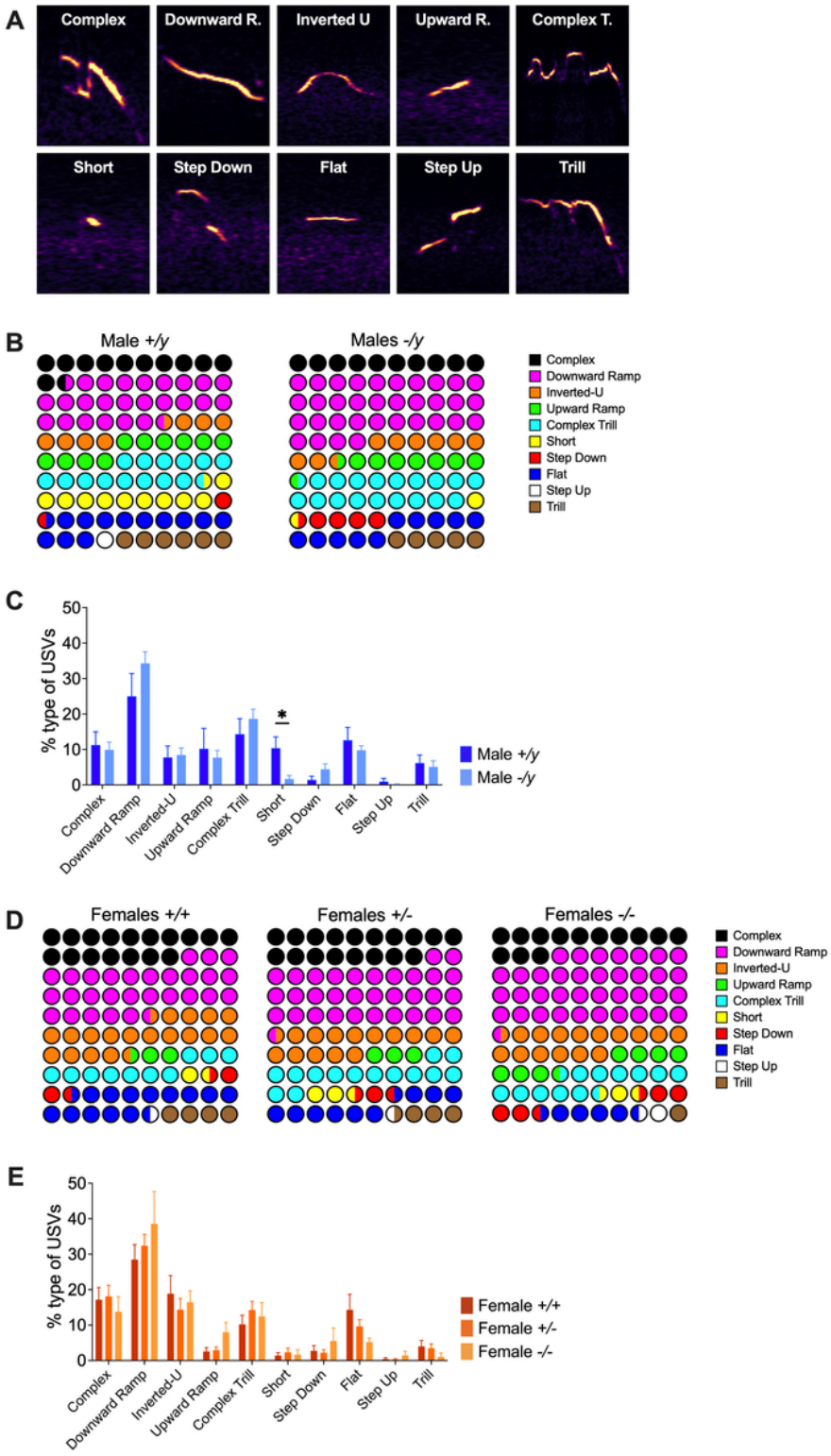

Figure 4

Vocal repertoire of FXS pups.

(**A**) Representative USVs calls classified into ten distinct categories based on a supervised-call classification neural network. (**B, C**) In the absence of FMRP, male mice exhibit a limited use of short calls in their vocal repertoire. (**D, E**) The vocal repertoire of female pups remains unaffected by the absence of

FMRP. (**B, D**) Data are represented as a percentage utilization of each category of USVs for each group. (**C, E**) Data are shown as a bar graph (± SEM) indicating the percentage utilization of each type of USV category for each group. Significance: * *p*-values <0.05, full statistical details can be found in Table 6. Sample sizes: (**B-E**) *+/y* males N=9, *-/y* males N=14, *+/+* females N=7, *+/-* females N=13 and *-/-* females N=6.

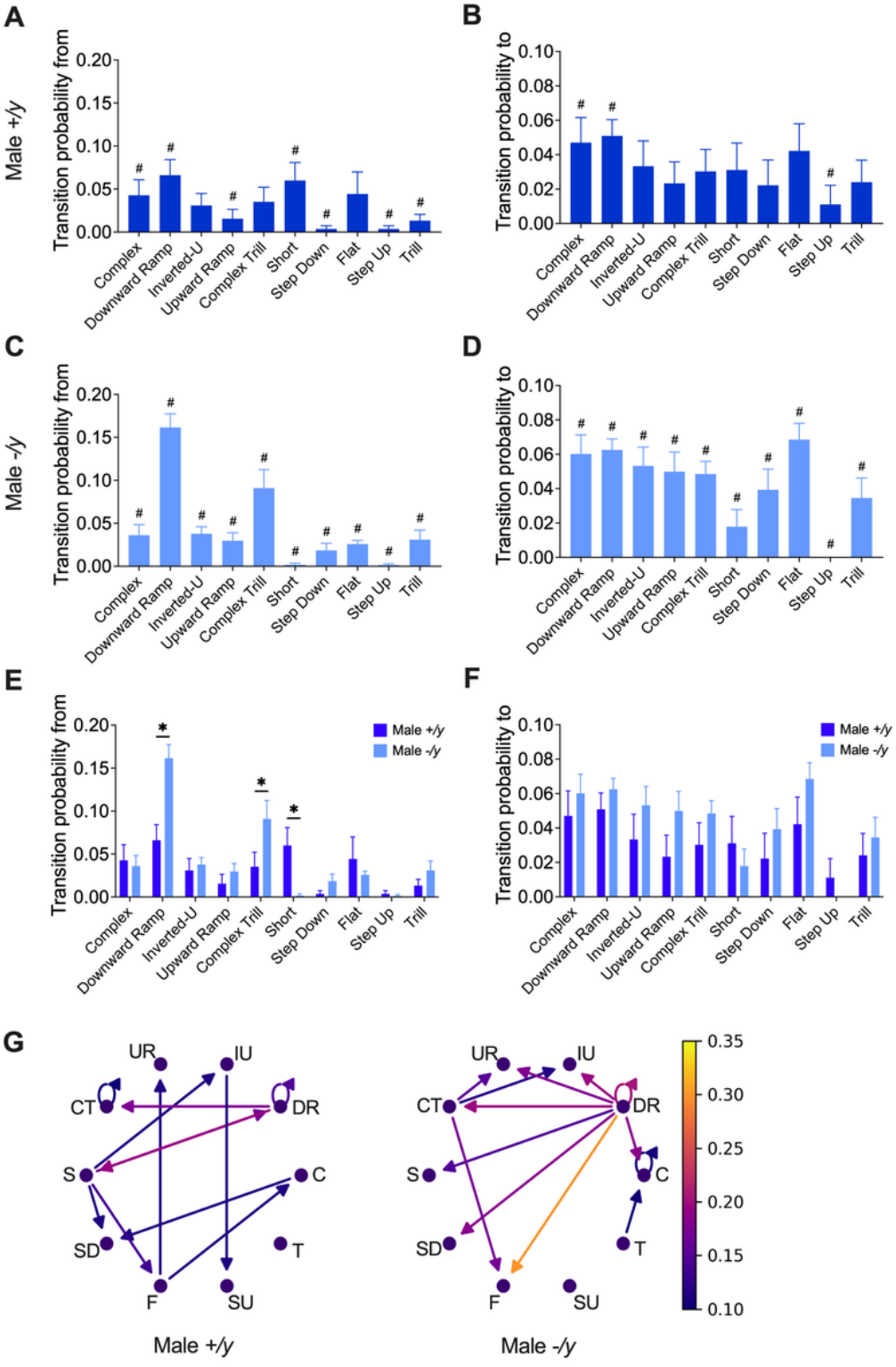

Figure 5

Syntactic transition probability in male FXS pups.

(A – D) The probability of transitions 'from' and 'to' a specific USV class varies among different vocalization classes in ***+/y* (A, B) and *-/y* (C, D) males.** (**E, F**) Transition probabilities differ 'from' a specific USV class (**E**), but not 'to' a specific class (**F**). (**G**) Qualitative illustration of transition probability profiles for males of various genotypes. (**A-F**). The data is displayed in bar graph format with error bars indicating the standard error of the mean (± SEM). The *p*-values less than 0.05 are indicated with an asterisk (*) and the full statistics can be found in Tables 9 and 12. The hashtag (#) refers to statistics presented in Tables 7 and 10. Statistical analysis was done using Mann-Whitney U tests. (**G**) Arrow diagrams. Arrows indicate transition directions, with brighter colors signifying higher transition probabilities. C=Complex, DR=Downward ramp, IU=Inverted-U, UR=Upward ramp, CT=Complex trill, S=Short, SD=Step Down, F=Flat, SU=Step up, and T=Trill. Sample sizes: (**A-G**) *+/y* males N=9 and *-/y*males N=14.

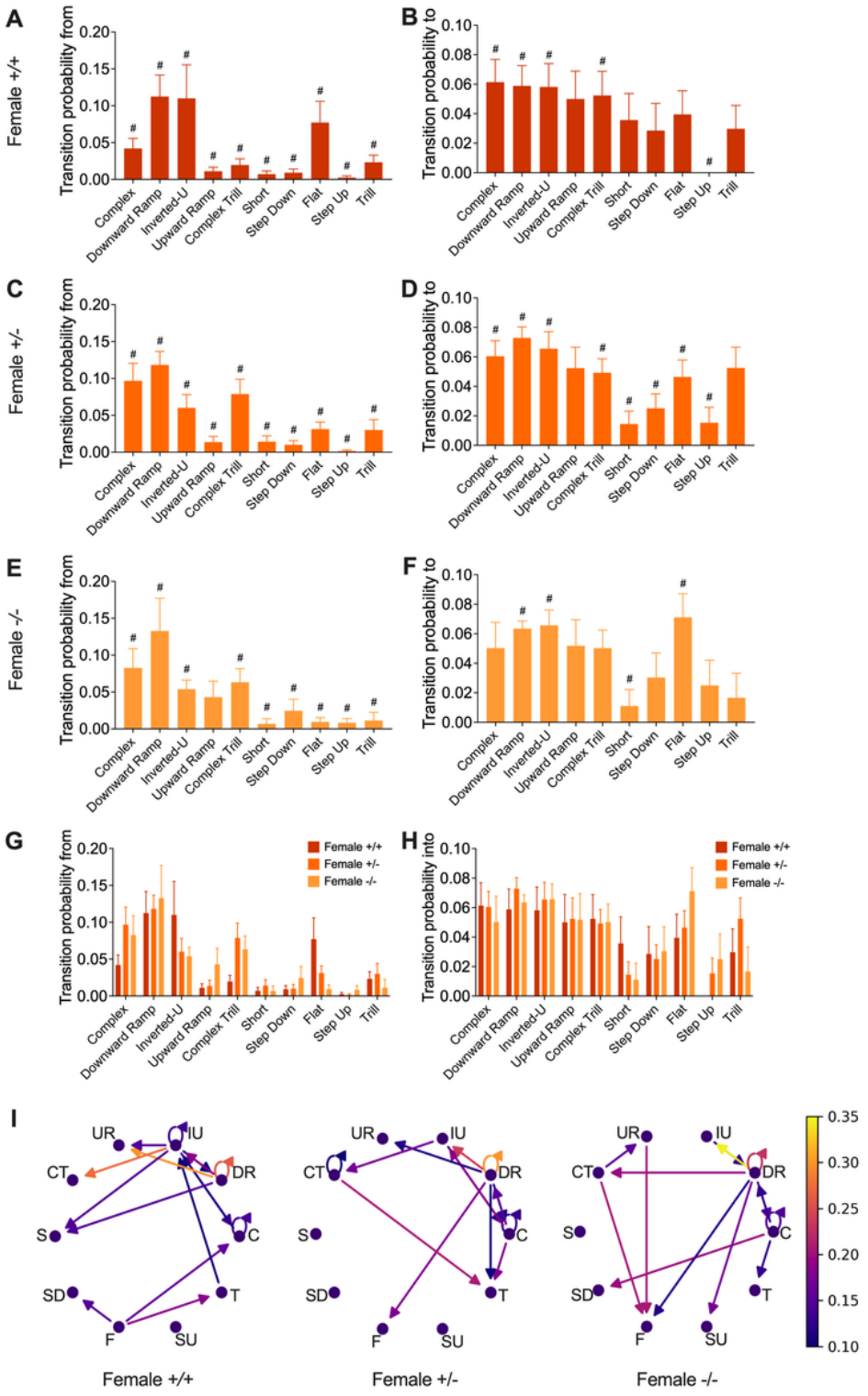

Figure 6

Syntactic transition probability in female FXS pups.

(A–F) The probability of transitions 'from' and 'to' a specific USV class varies among different vocalization classes **in *+/+* (A, B),** *+/-* (C, D) and *-/-* females (E, F). (G, H) These profiles are similar among genotypes in the probability of transition "from" (G) and "to" (H) a specific class of USVs. **(I)** Qualitative

illustration of transition probability profiles for females of various genotypes. **(A–H)** The data is displayed in bar graph format with error bars indicating the standard error of the mean (± SEM). (**A-F**) The hashtag (#) refers to statistics presented in Tables 8 and 11. (**G, H**) Full statistics can be found in Tables 9 and 12. (**A-H**) Statistical analysis was done using Mann-Whitney U tests. (**I**) Arrow diagrams. Arrows indicate transition directions, with brighter colors signifying higher transition probabilities. C=Complex, DR=Downward ramp, IU=Inverted-U, UR=Upward ramp, CT=Complex trill, S=Short, SD=Step Down, F=Flat, SU=Step up, and T=Trill. **Sample size:** (**A-I**) *+/+* females N=7, *+/-* females N=13 and *-/-* females N=6.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.tiff
- SupplementaryFigure2.tiff
- SupplTable1.docx
- SupplTable2.docx
- SupplTable3.docx
- SupplTable4.docx
- SupplTable5.docx
- SupplTable6.docx
- SupplTable7.docx
- SupplTable8.docx
- SupplTable9.docx
- SupplTable10.docx
- SupplTable11.docx
- SupplTable12.docx