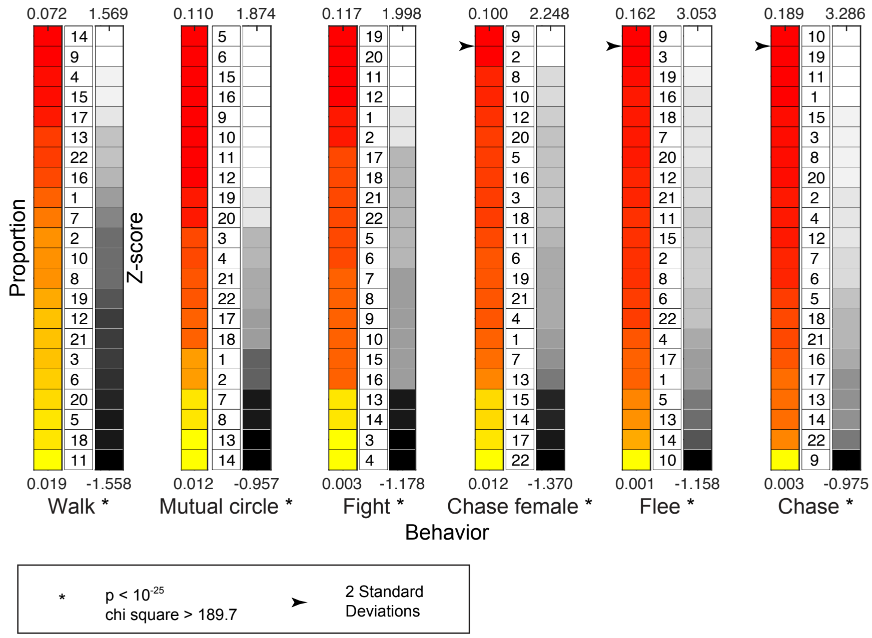
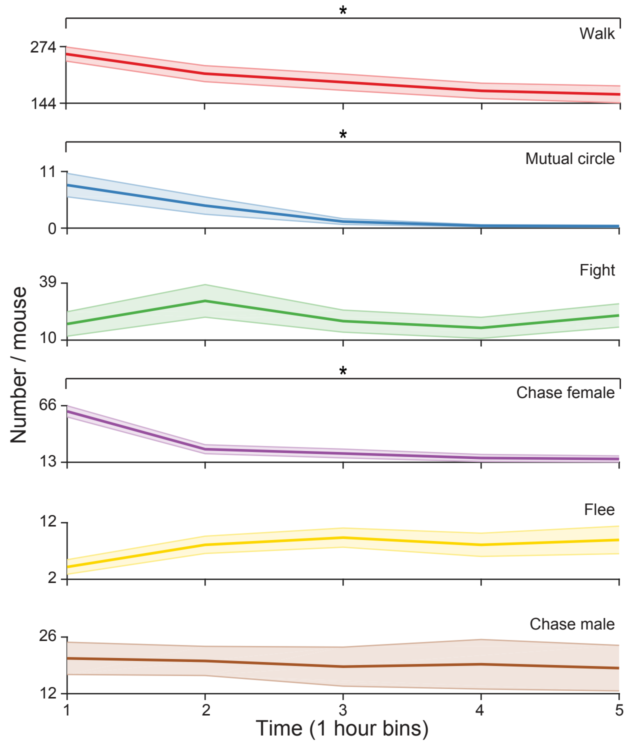
Supplementary figures

Supplementary Figure 1



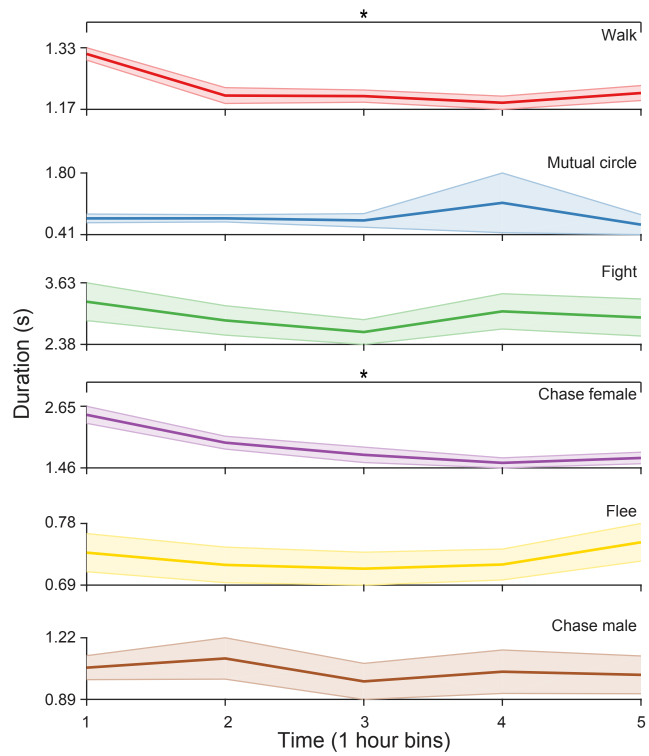
Supplementary Fig. 1 The number of times each behavior was performed varied across individuals. Rank distributions of all individuals (middle column) performing specified behavior. Behaviors denoted below bars. Left columns (warm colors) show proportion of times an individual performed the behavior. Proportion was calculated by dividing the number of times that a particular male performed a specific behavior by the total number of times the behavior was performed by all the males. Top indicates highest proportion and bottom shows lowest proportion. Right columns show z-scores (gray scale). Top shows highest z-score and bottom indicates lowest z-score. There were 22 biologically independent samples in each behavior, except for fight and mutual circle where there were 11 samples (all X^2>189.7, all $p<10^{-25}$).

Supplementary Figure 2



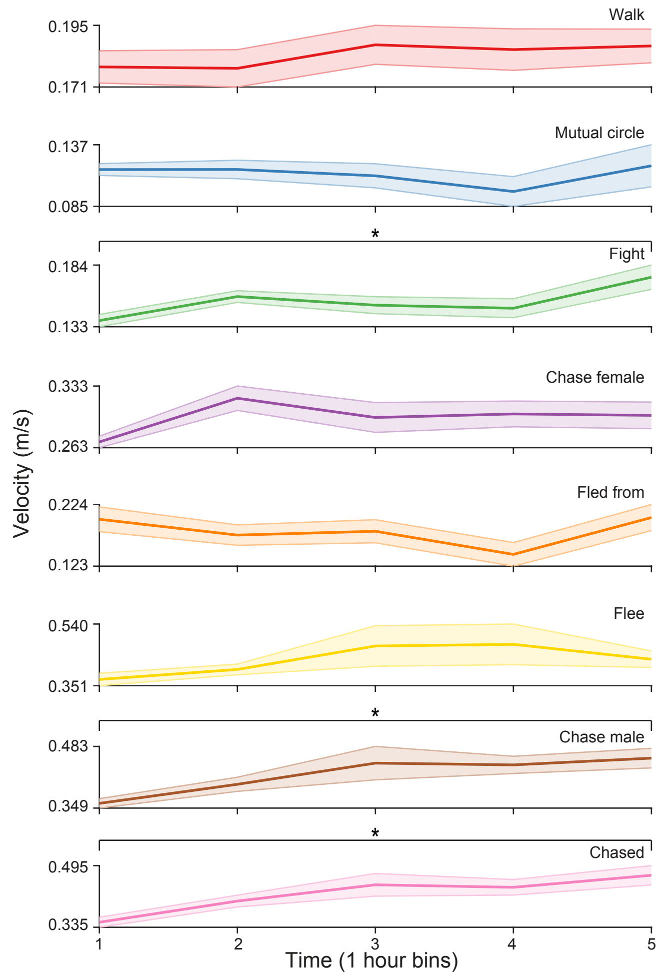
Supplementary Fig. 2. Temporal profile of behavior. Frequency that male mice performed each behavior over time (Average±SEM; n=110 biologically independent samples for each behavior, * = temporal differences, 1-way ANOVA, all F>4.0, all p<0.005). Occurrences binned hourly.

Supplementary Figure 3



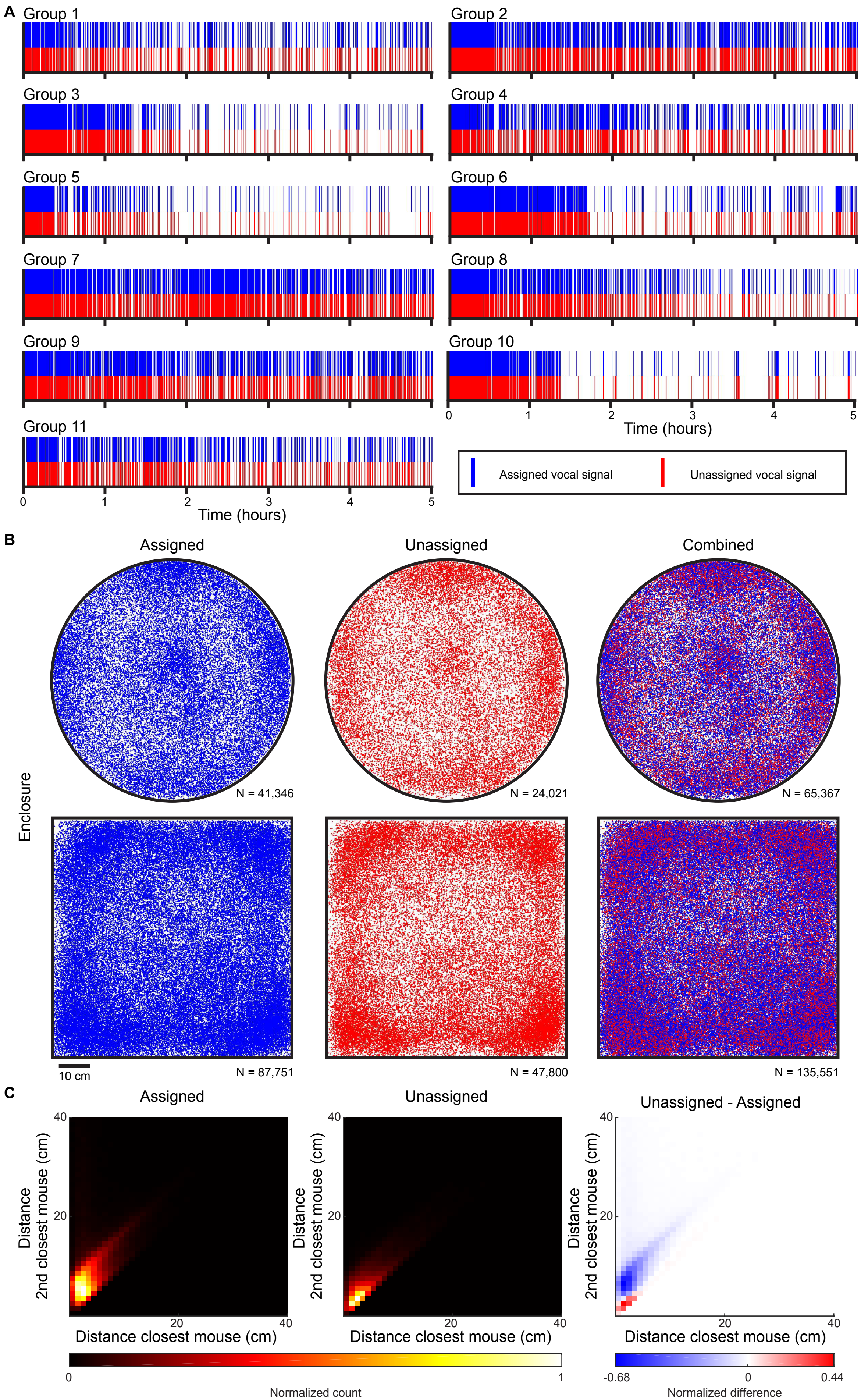
Supplementary Fig. 3. Temporal profile showing duration of behaviors. The average duration of each behavioral event was calculated for every male mouse and binned hourly (Average±SEM; there were 110 (walk), 32 (mutual circle), 51 (fight), 110 (chase female), 94 (flee), and 103 (chase male) biologically samples independently analyzed, * = temporal differences, 1-way ANOVA, all significant $F>8.0$, all significant $p<10^{-5}$).

Supplementary Figure 4



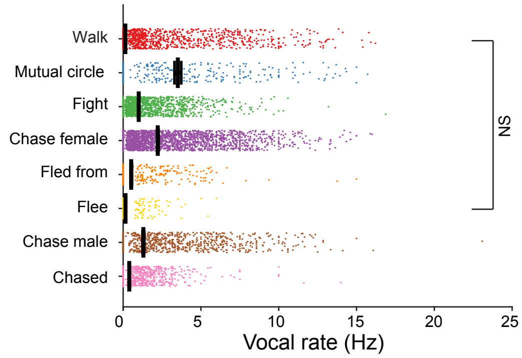
Supplementary Fig. 4. Temporal profile showing velocity of animals during each behavior. The speed that every male mouse was moving during each behavioral event was computed, binned hourly, and then averaged across mice (Average ± SEM; there were 110 (walk), 64 (mutual circle), 102 (fight), 110 (chase female), 94 (fled from), 94 (flee), 103 (chase male), and 103 (chased) biologically samples independently analyzed, * = temporal differences, 1-way ANOVA, all significant $F>3.4$, all significant $p<0.05$).

Supplementary Figure 5



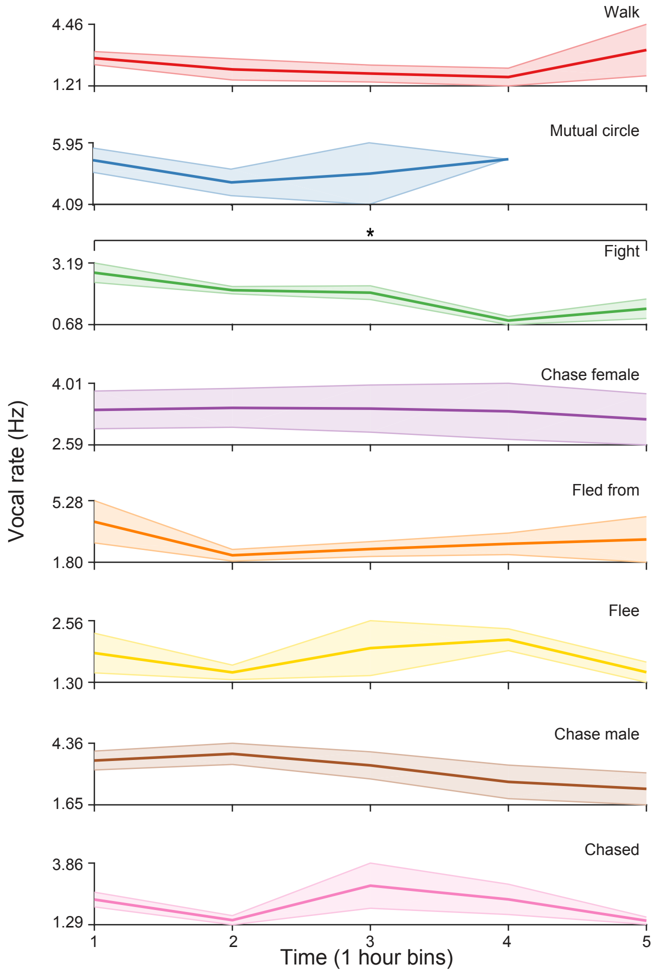
Supplementary Fig. 5. Patterns in assigned and unassigned vocal signals. A, Assigned (blue) and unassigned (red) vocal signals plotted as a function of time. In all groups, assigned and unassigned vocal signals occur at similar times throughout the experiment. **B,** Assigned (blue) and unassigned (red) vocal signals were distributed throughout the circular and square enclosures. Combined shows the overlay between assigned and unassigned signals. **C,** Peak-normalized heat maps showing distance between the estimated sound source location and the two closest mice for assigned (left) and unassigned (right) vocal signals. Taking the difference between unassigned and assigned maps revealed that vocal signals are predominantly assigned when the estimated sound source was closest to one mouse, but unassigned when two mice were equidistant from the estimated sound source.

Supplementary Figure 6



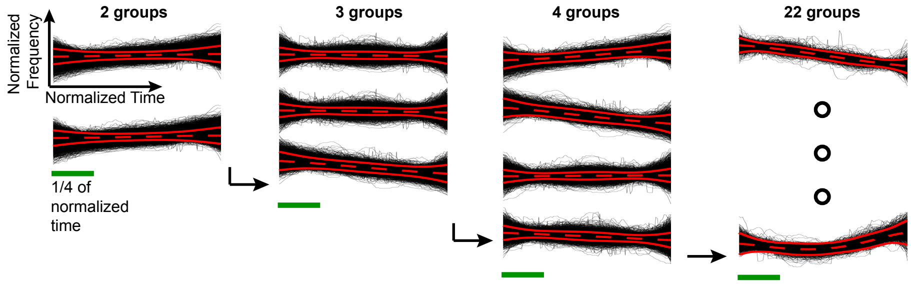
Supplementary Fig. 6. Rate of vocal emission varies between behaviors. Vocal rate of the animals performing each behavior. Black vertical line = average, width of line = ±SEM, n=33,692 biologically independent samples, 1-way ANOVA, F=590.7, $p<10^{-100}$, 2-sided Fisher's least significant difference and corrected for multiple comparisons using the Benjamini-Hochberg procedure, all significant T≤-2.05 or T≥3.99, all significant p<0.05, NS = only non-significant comparison, T=0.19, p=0.84.

Supplementary Figure 7



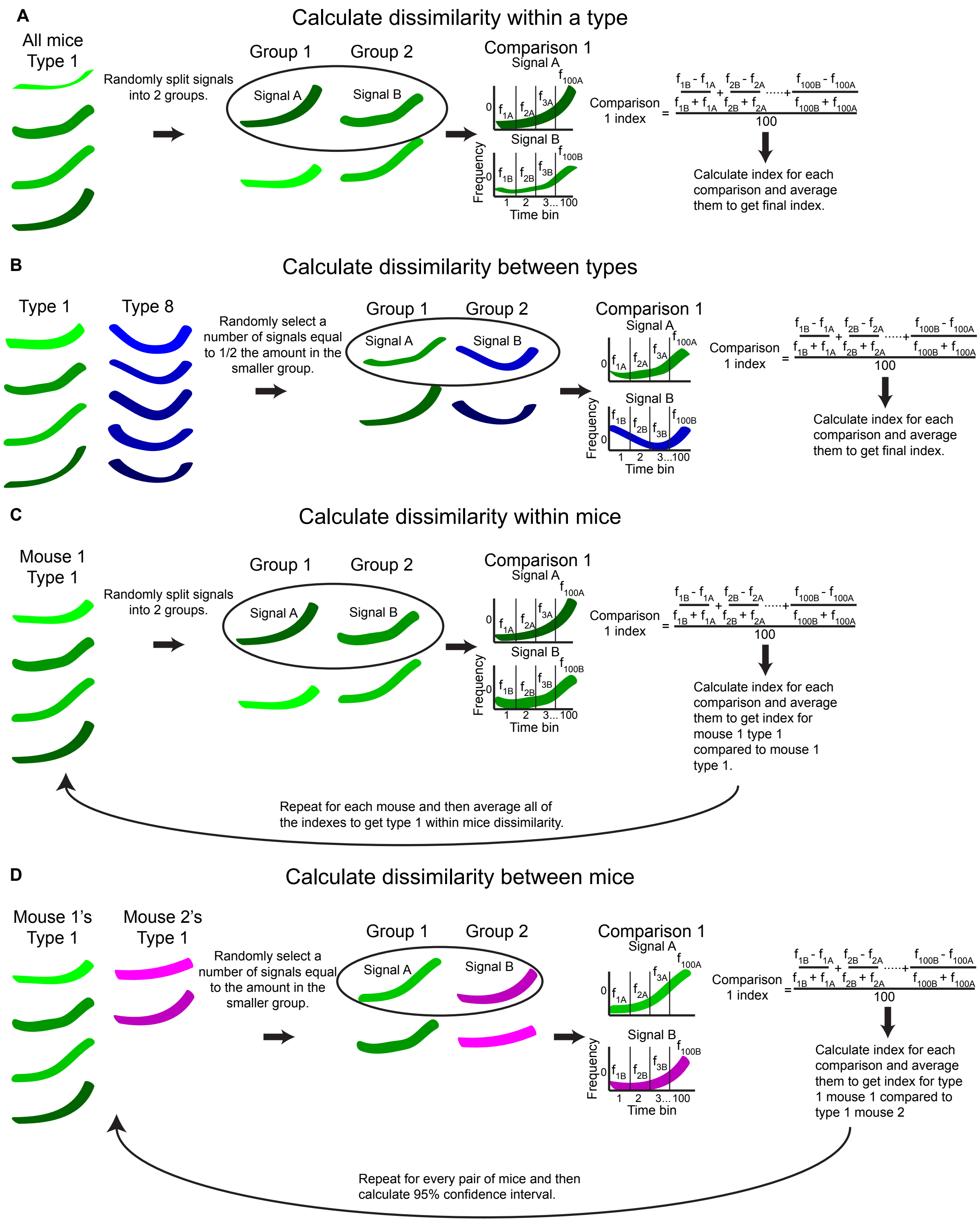
Supplementary Fig. 7. Temporal profile showing vocal rate of animals during each behavior. The vocal rate of every male mouse during each behavioral event was computed, binned hourly, and averaged across mice (Average±SEM; there were 58 (walk), 38 (mutual circle), 68 (fight), 96 (chase female), 40 (fled from), 28 (flee), 57 (chase male), and 57 (chased) biologically samples independent analyzed, * = temporal differences, 1-way ANOVA, all significant F>5.0, p<0.01). Note that no mutual circles with assigned vocalizations were detected in the last hour of any experiment.

Supplementary Figure 8



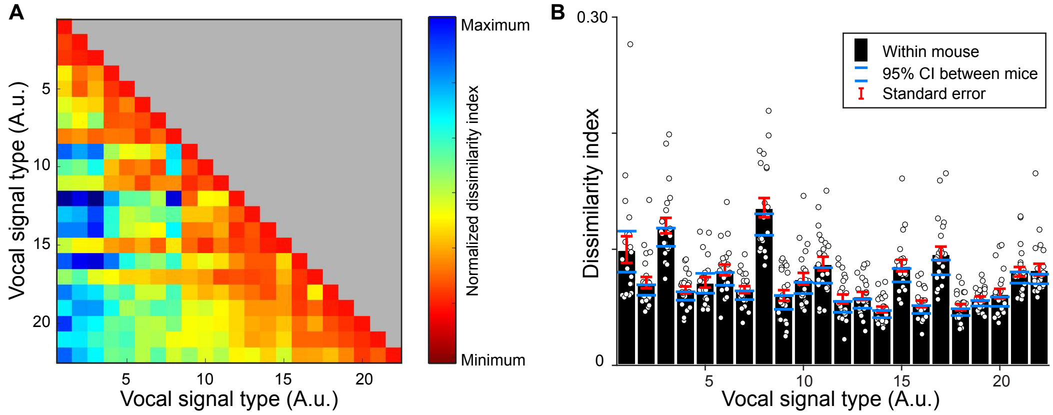
Supplementary Fig. 8. Automatic, progressive vocal signal clustering. Signals were first normalized in duration and frequency and then clustered into two groups based on the shape of their frequency contours using K-means clustering. Next, the mean (hashed red line) and standard deviation were calculated for each time bin in each group. If more than 25% of the time bins (indicated by green horizontal bars) for more than 3% of the vocal signals within a group were outside of 2.5 standard deviations from the mean (solid red lines), all of the signals were re-clustered using one more cluster than the previous iteration. This process was repeated until 97% of all vocal signals in each cluster had at least 75% of their time bins within 2.5 standard deviations of the mean. A total of 211,090 vocal signals were clustered.

Supplementary Figure 9



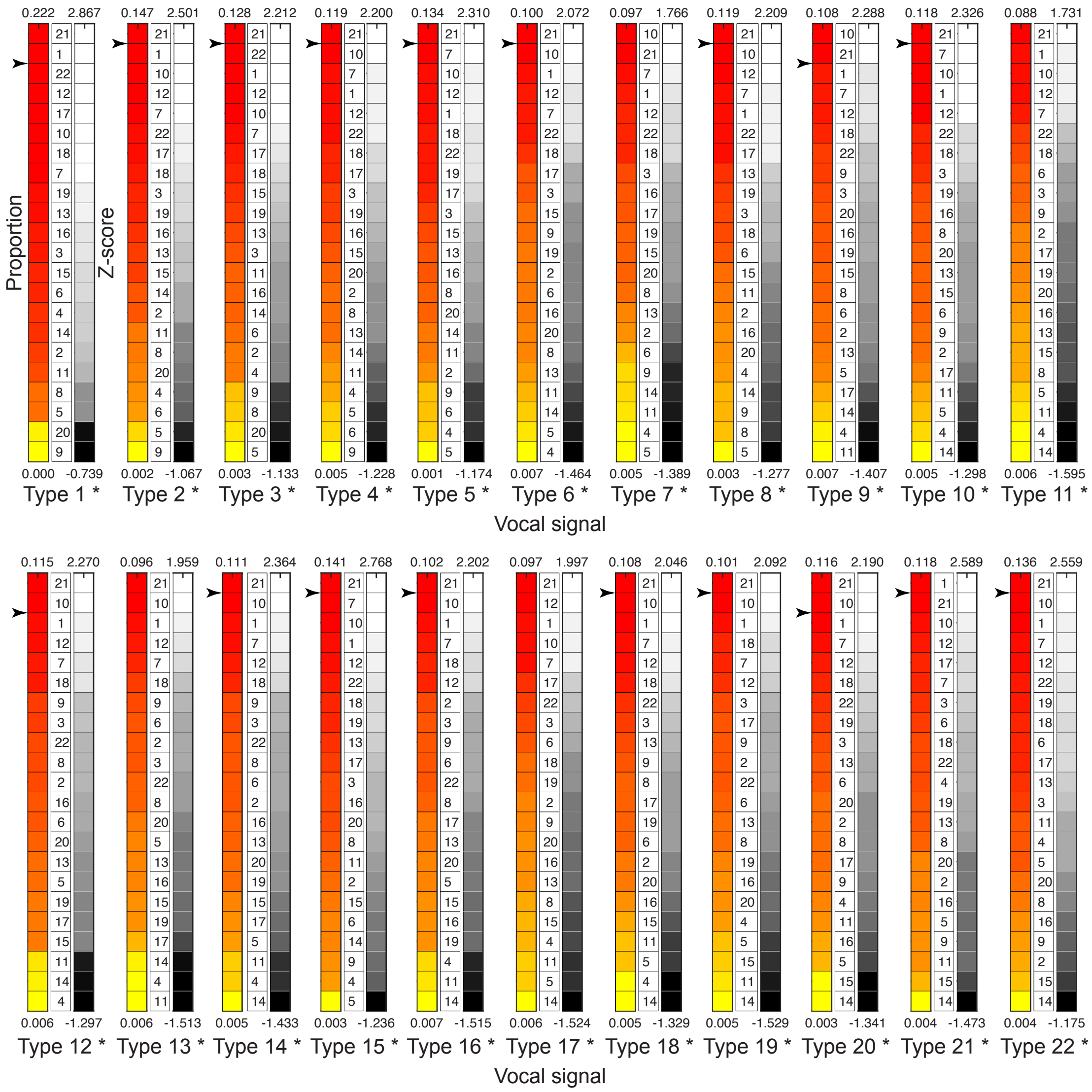
Supplementary Fig. 9. Schematics for calculating dissimilarity between vocal signals. A, Dissimilarity between signals of the same type. **B,** Dissimilarity between signals of different types. **C,** Dissimilarity between signals of the same type emitted by the same mouse. **D,** Dissimilarity between signals of the same type emitted by different mice.

Supplementary Figure 10



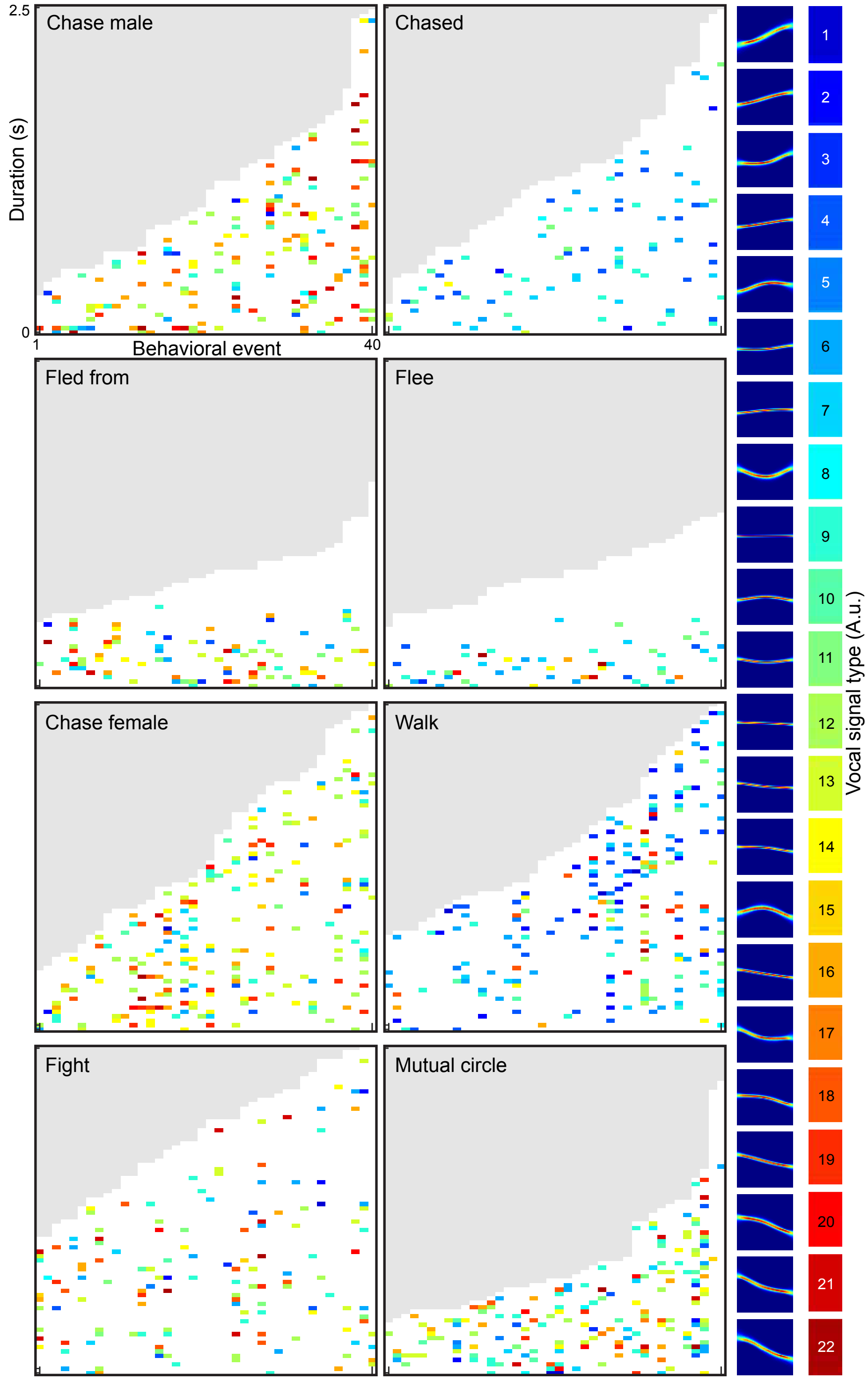
Supplementary Fig. 10. Dissimilarity between vocal signal types. A, A dissimilarity index was calculated to quantify the uniformity between each of the 22 types of vocal signals. Signals of the same type were more similar to each other than they were to other types (i.e., diagonal band representing the identity line). Indices denoting maximum and minimum dissimilarity are indicated by blue and red, respectively. Heat map was scaled to the peak difference. **B,** Index comparing the phonetic similarity of vocal signals produced by an individual mouse to signals of the same type produced by other mice. Scores close to 1 and 0 indicate larger and smaller spectral differences. Red lines = average±SEM; n=481 biologically independent samples analyzed (22 samples per vocal signal type, except for type 1 and 5 in which some mice did not emit these types of signals).

Supplementary Figure 11



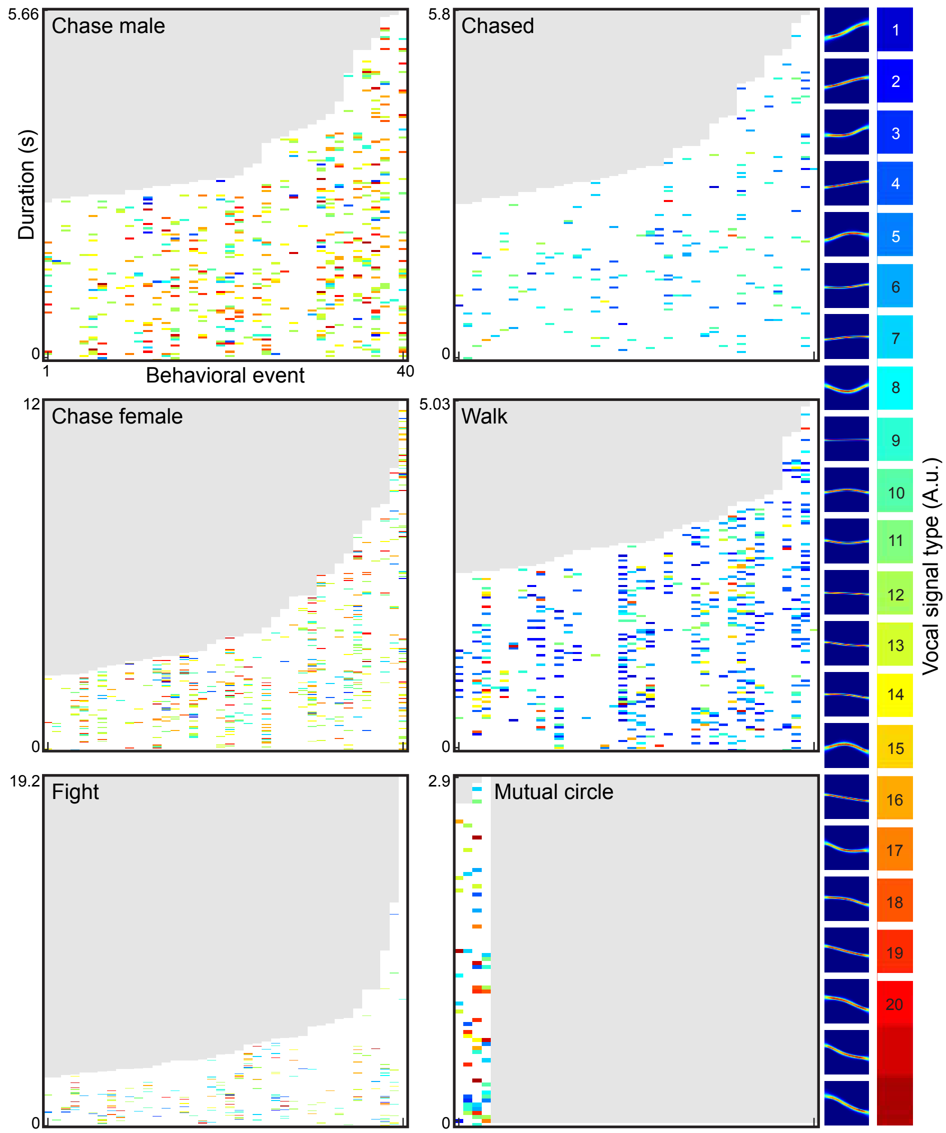
Supplementary Fig. 11. The number of times each type of vocal signal was emitted varied across individuals. Rank distributions of all individuals (middle column) emitting specified vocal signal types. Vocal signal type denoted below bars. Left columns (warm colors) show proportion of times an individual emitted a specific type of vocal signal. Proportion was calculated by dividing the number of times that a particular male emitted a specific type of vocal signal by the total number of times the vocal signal type was emitted by all the males. Top indicates highest proportion and bottom shows lowest proportion. Right columns show z-scores (gray scale). Top shows highest z-score and bottom indicates lowest z-score. There were 484 biologically independent samples analyzed. * = all $\chi^2>603$ and all $p<10^{-114}$, arrow denotes 2 standard deviations above the mean).

Supplementary Figure 12



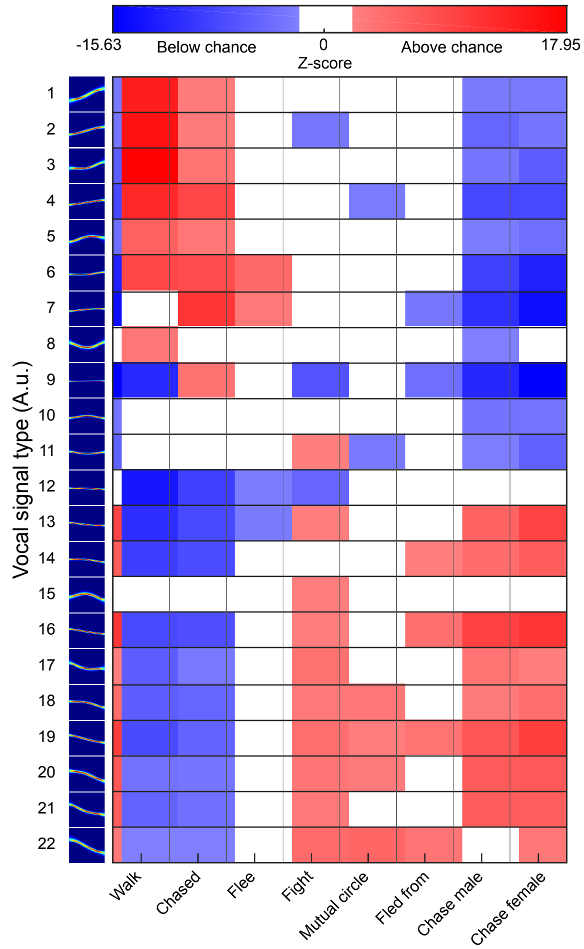
Supplementary Fig. 12. Social context-dependent vocal expression. Instances of each behavior are shown in white and indicated on the x-axis. Randomly selected examples of each behavior that contained vocal signals and were shorter than 2.5 seconds. Behaviors lasting less than 2.5 seconds were chosen to facilitate the ability to visualize the types of vocal signals emitted in specific behavioral contexts. Vocal signal types are indicated by uniquely colored rectangles. Spectrograms show the vocal signal type.

Supplementary Figure 13



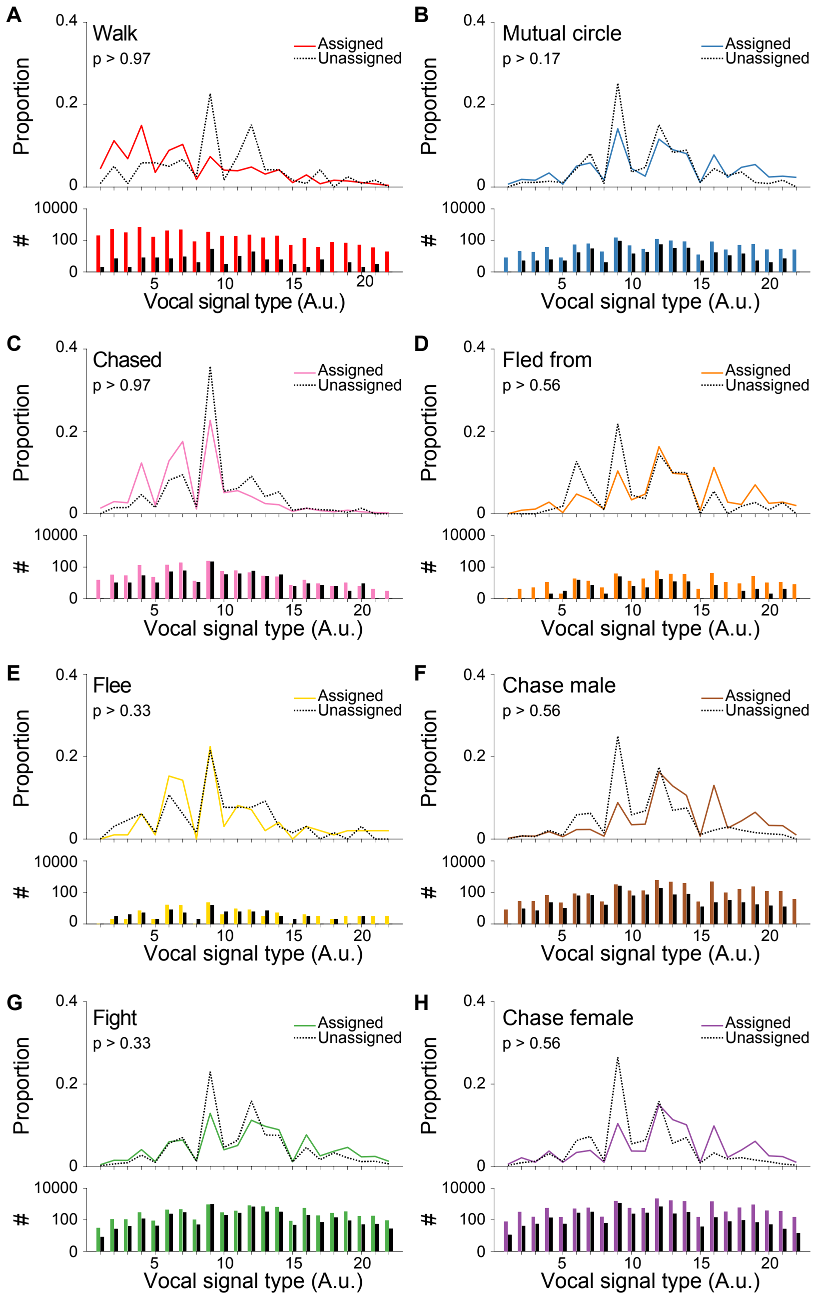
Supplementary Fig. 13. Social context-dependent vocal expression occurring in behaviors lasting longer than 2.5 seconds. For display purposes in **Supplementary Figure 10**, example behaviors lasting less than 2.5 seconds were chosen to facilitate the ability to visualize the types of vocal signals emitted in specific behavioral-contexts. For behavioral examples lasting longer than 2.5 seconds, a similar pattern was observed. Instances of each behavior are shown in white and indicated on the x-axis. A maximum of 40 examples that were longer than 2.5 seconds and contained vocal signals were randomly selected for each behavior. Mutual circle only had four examples lasting longer than 2.5 seconds—all of which are shown. Note that there were no flees that lasted longer than 2.5 seconds. Vocal signal types are indicated by uniquely colored rectangles. Spectrograms show the vocal signal type**.**

Supplementary Figure 14



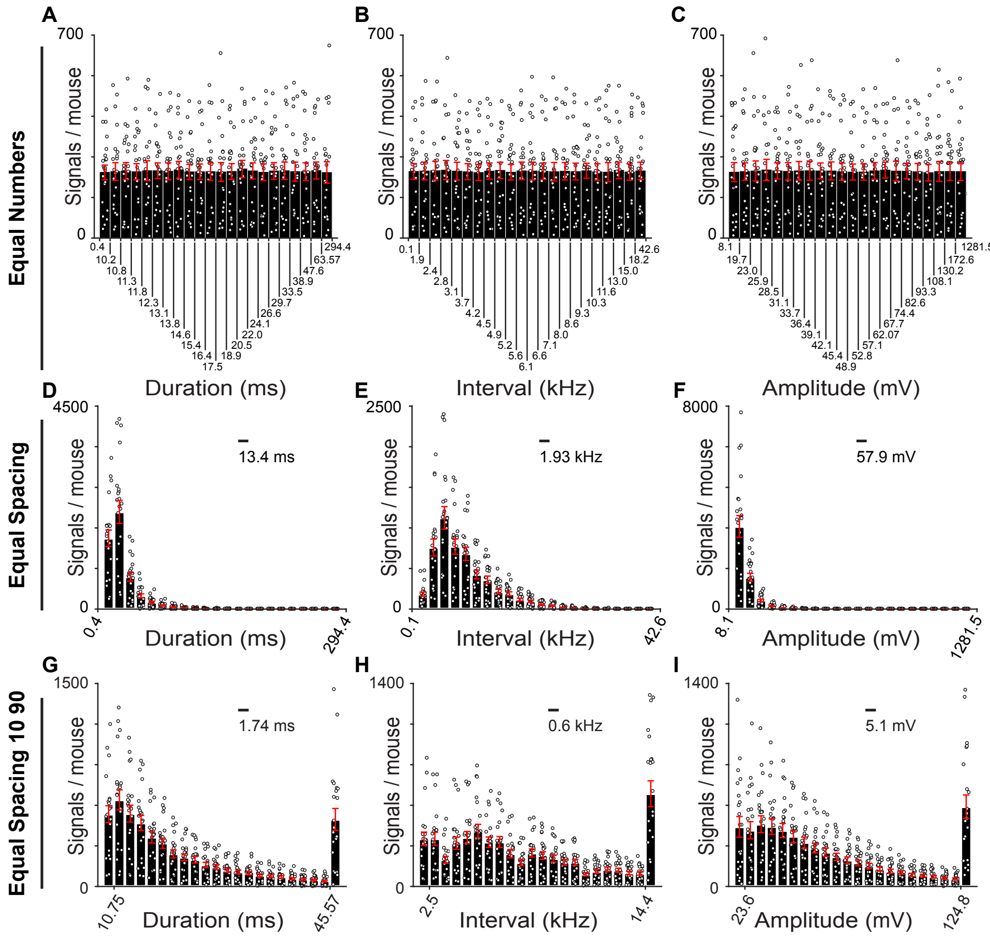
Supplementary Fig. 14. Proportion of behavioral events with specific types of vocal signals. Permutation analysis used to determine chance levels of simultaneously occurring behavior and vocal signal type emission. Different types of signals were emitted above (red), below (blue), and at chance (white) in different behaviors. Note the similarity to chance levels calculated based on the proportion of signal expression (**Fig. 6A**). 2-sided permutation tests, n=1000 independent permutations.

Supplementary Figure 15



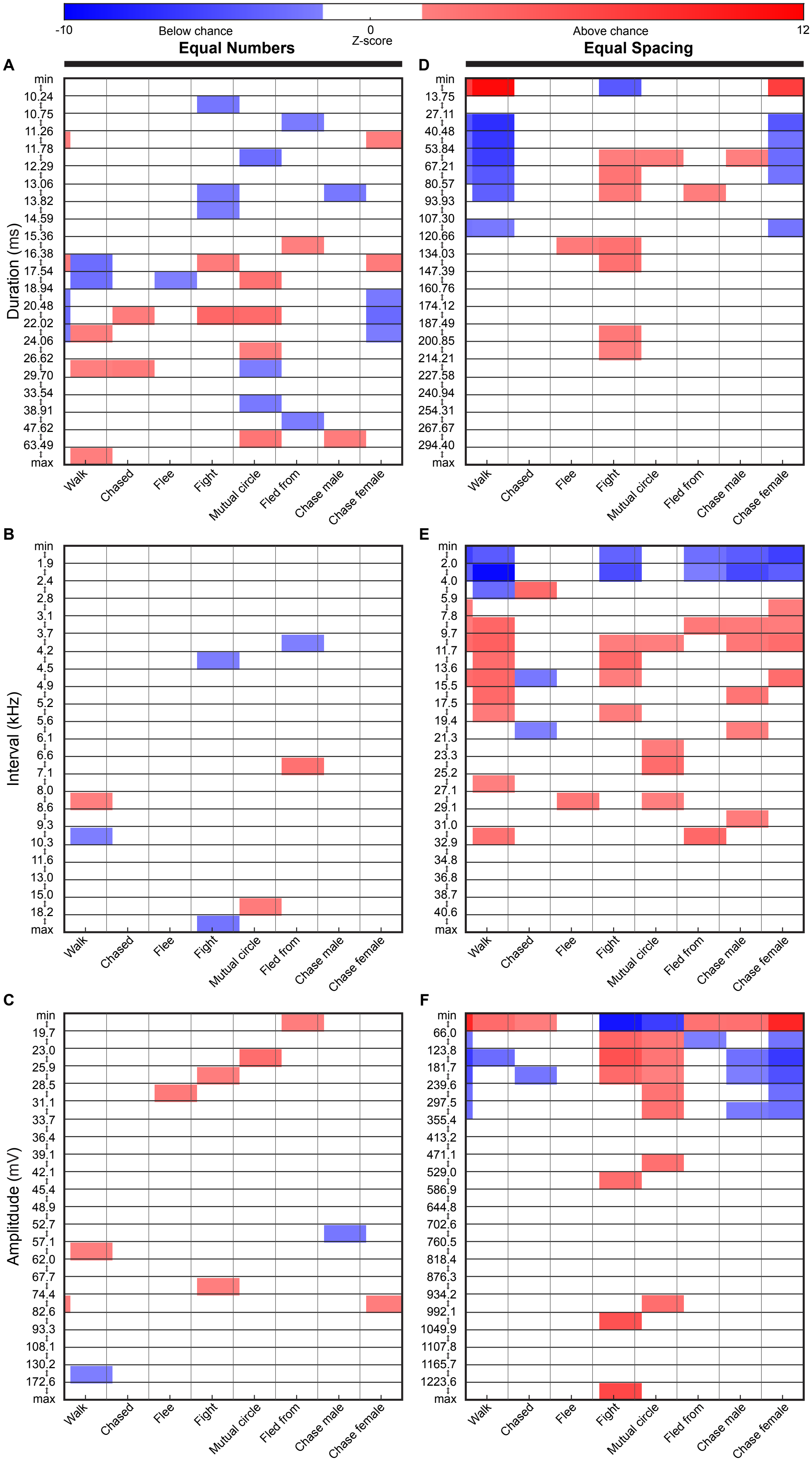
Supplementary Fig. 15. Potential assignment bias during behaviors. A-H, For every behavior, the proportion of each assigned signal type was calculated. For this analysis, unassigned signals were assigned to the animal that was most likely to have emitted the signal (i.e. the animal with the highest MPI value [see online methods]). After assigning the unassigned signals, the proportion of each unassigned signal type was calculated for each behavior. Note that there were no significant differences between assigned and unassigned signal types within each behavior (n=44 biologically independent samples for each behavior analyzed, two-sided 2-sample Kolmogorov–Smirnov test, all D<0.4, all p>0.17), suggesting that the omission of unassigned signals from the analyses are unlikely to alter the results.

Supplementary Figure 16



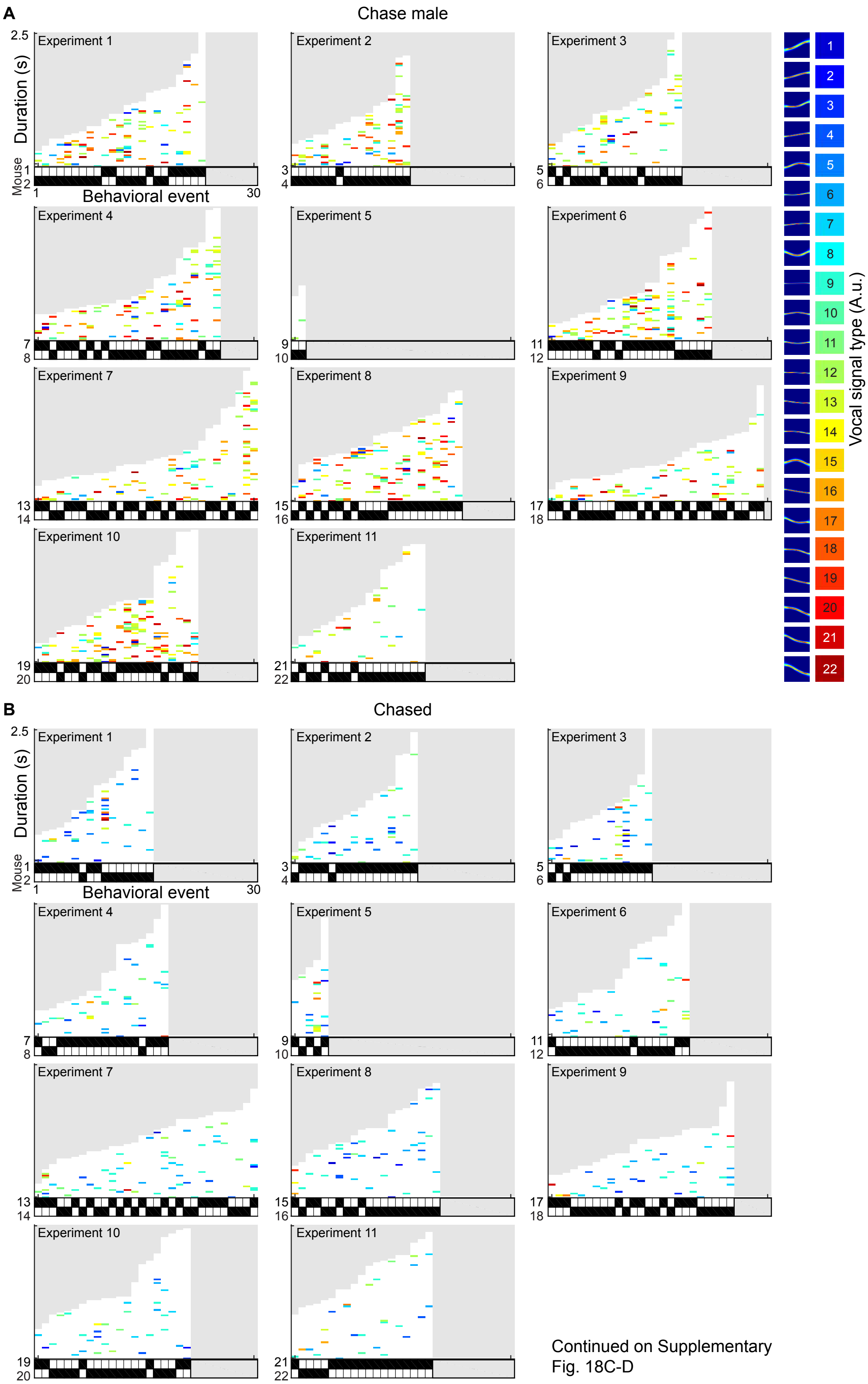
Supplementary Fig. 16. Categorization based on spectral features. A-C, Distributions of signals categorized based on duration, interval, and amplitude with equal numbers of signals in each category. To ensure that each category had an equal number of vocal signals, the total number of assigned signals was divided by 22, which matched the number of shape categories, and then boundaries were defined by rank ordering the signals based on the specified vocal attributes. **D-F,** Signals were categorized based on duration, interval, and amplitude by creating 22 equally spaced bins. **G-I**, Signals were categorized based on duration, interval, and amplitude by creating 20 equally spaced bins and the bottom and top 10% of all vocal signals were put into their own respective categories. Bottom 10% are classified as category 1 and top 90% are classified as category 22. Similar to signal type usage, the duration, interval, and amplitude of signals were highly variable regardless of the boundary edges used for categorizing the signals (1-way ANOVA, all F>158.0, all $p<10^{-100}$). **A-J**, Red line = average, width of line = ±SEM; there were 484 biologically independent samples included in each analysis.

Supplementary Figure 17

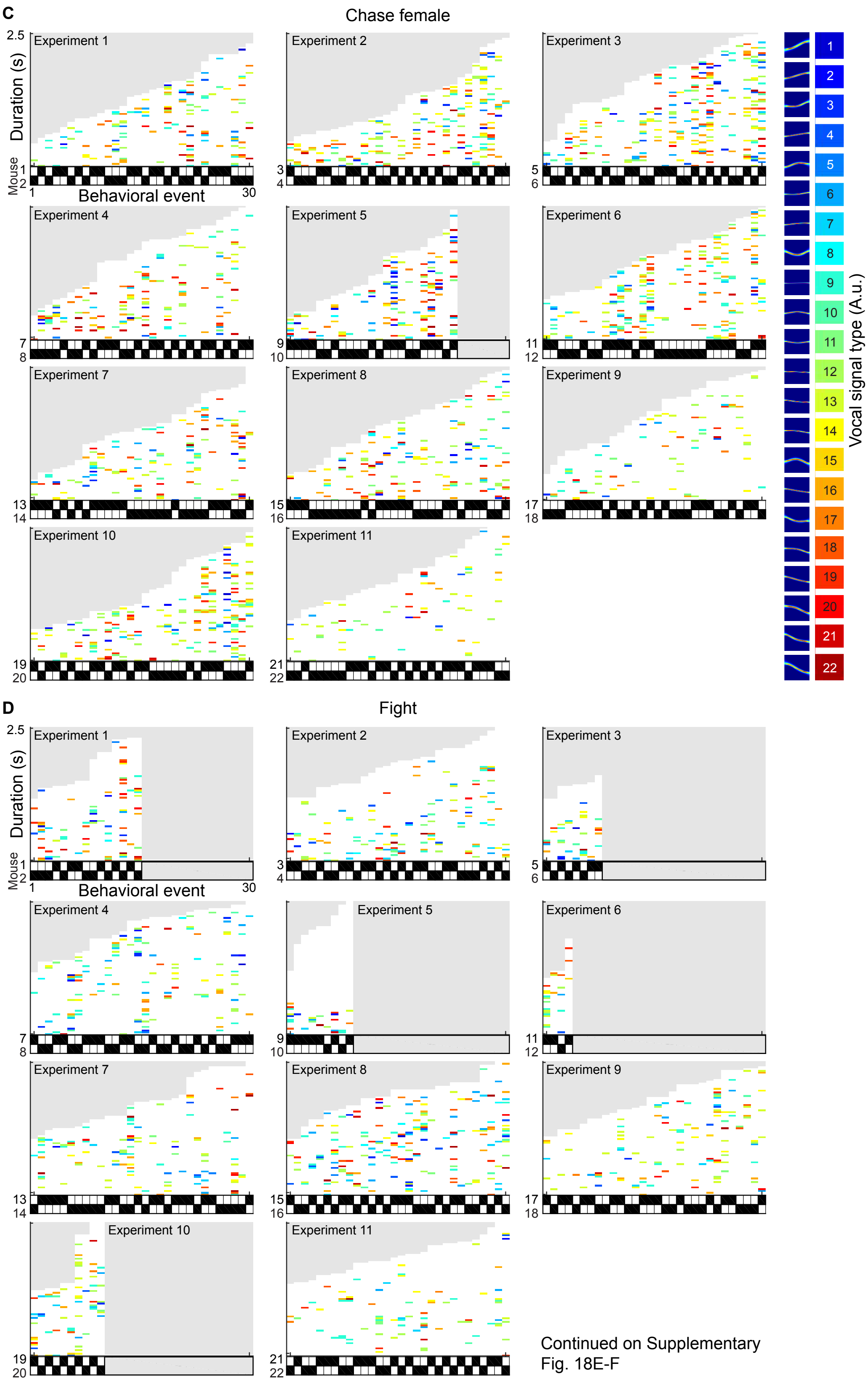


Supplementary Fig. 17. Vocal signal expression during behavior using alternative approaches for categorizing duration, interval, and amplitude. Different categories of signals were emitted above (red), below (blue), and at chance (white) in different behaviors. Categorizing based on equal numbers (left column, **A, B, C**) and equal spacing (right column, **D, E, F**). **A,D,** Duration. **B,E,** Interval, **C,F,** Amplitude. **A-F,** 2-sided permutation tests, n=1000 independent permutations for each analysis.

Supplementary Figure 18



Continued on Supplementary Fig. 18C-D



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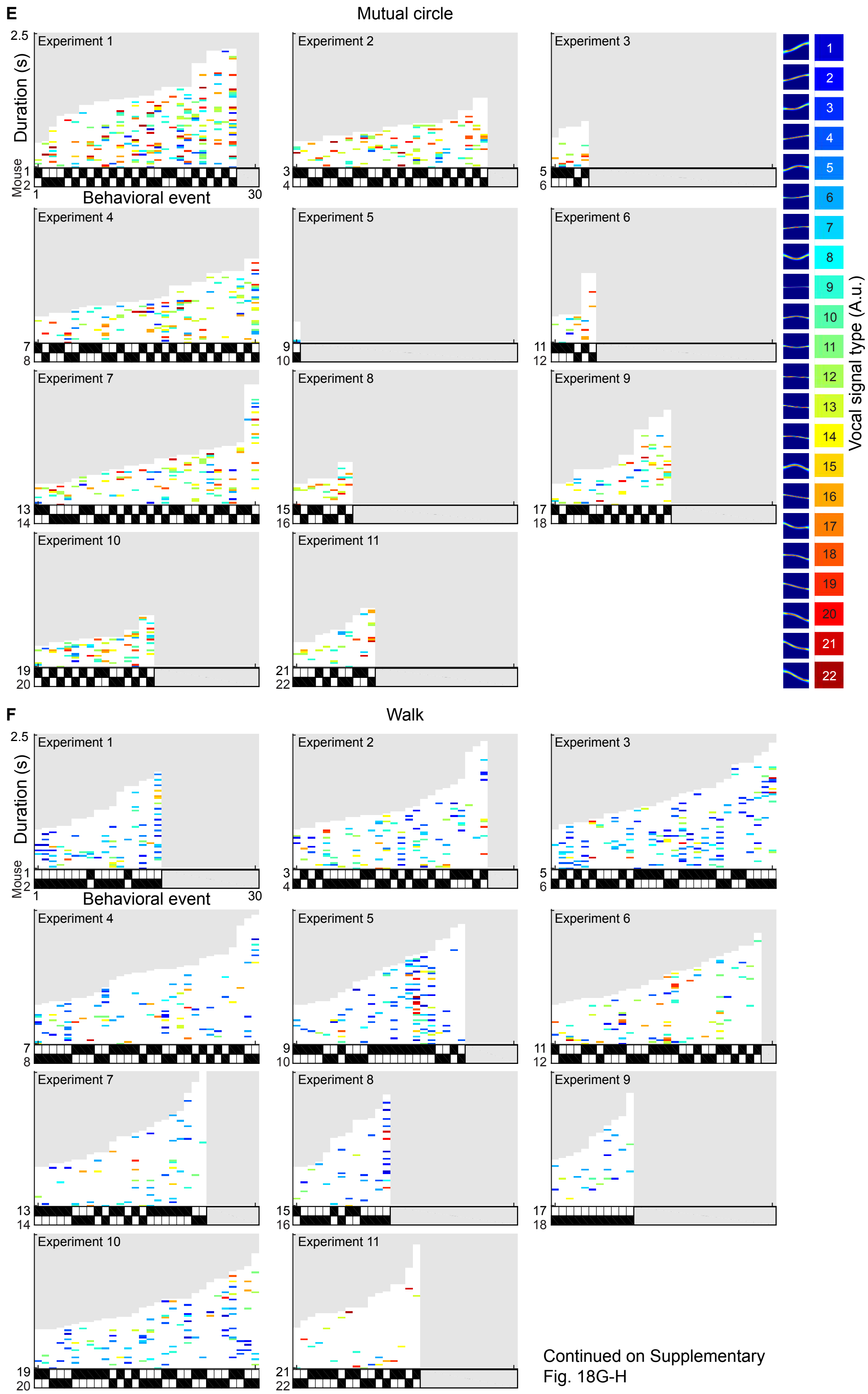

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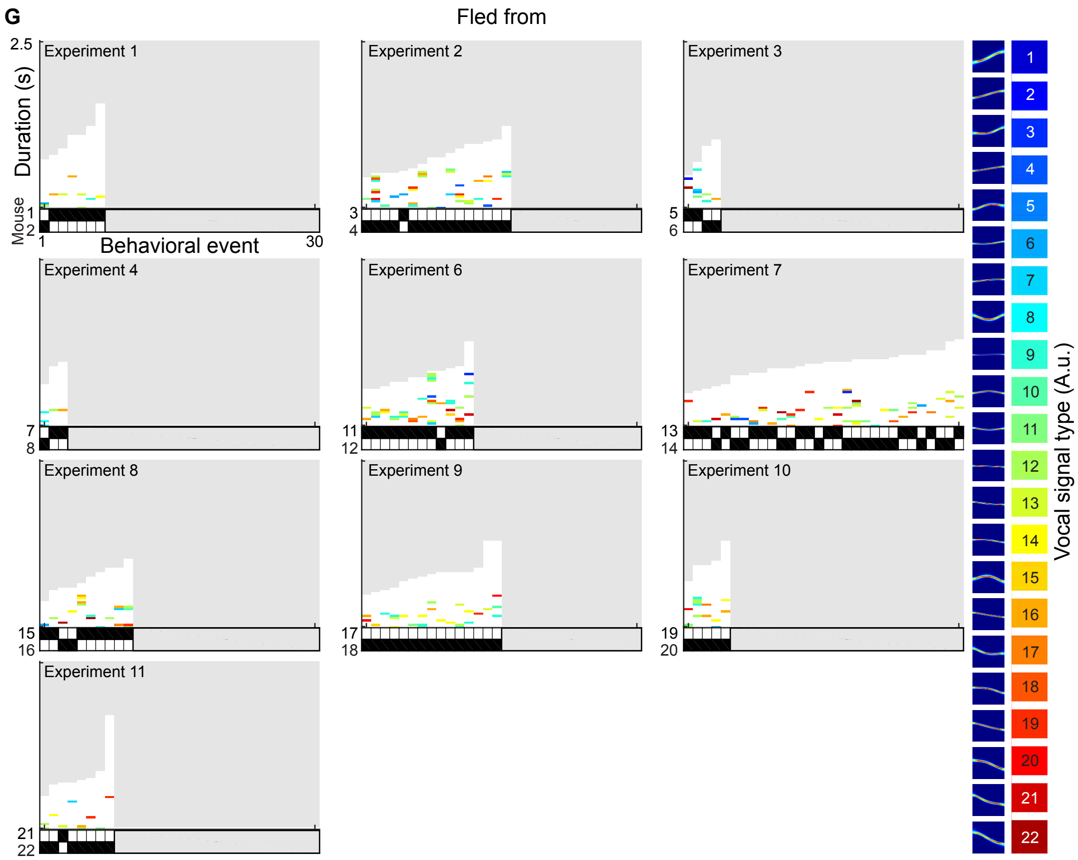
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Duration (s)
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Vocal signal type (A.u.)
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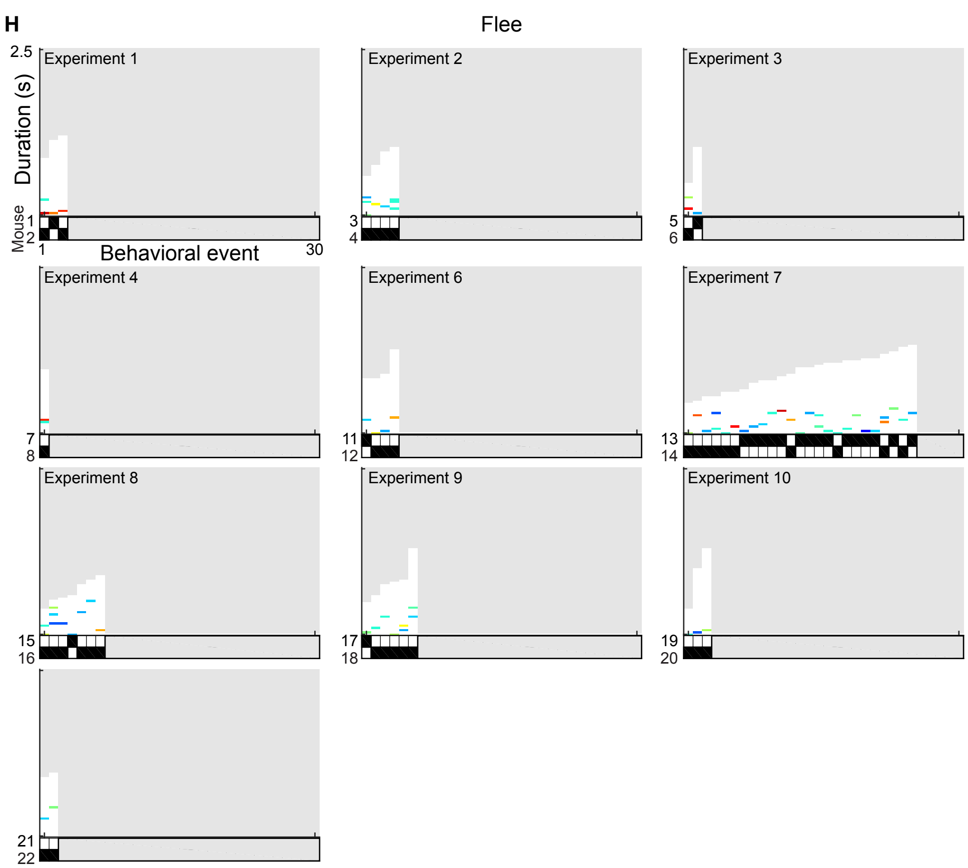
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Duration (s)
Mouse
Behavioral event
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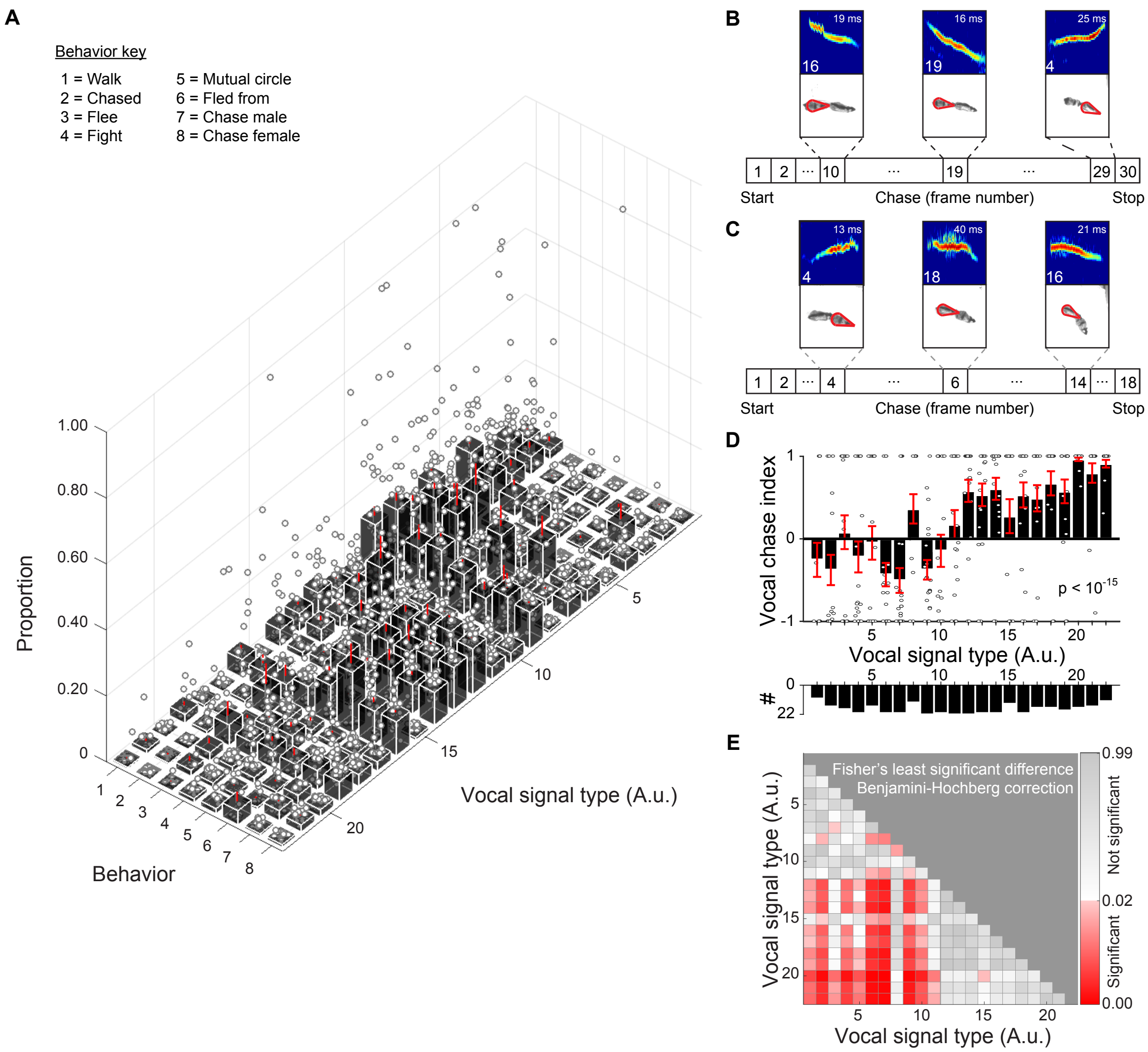
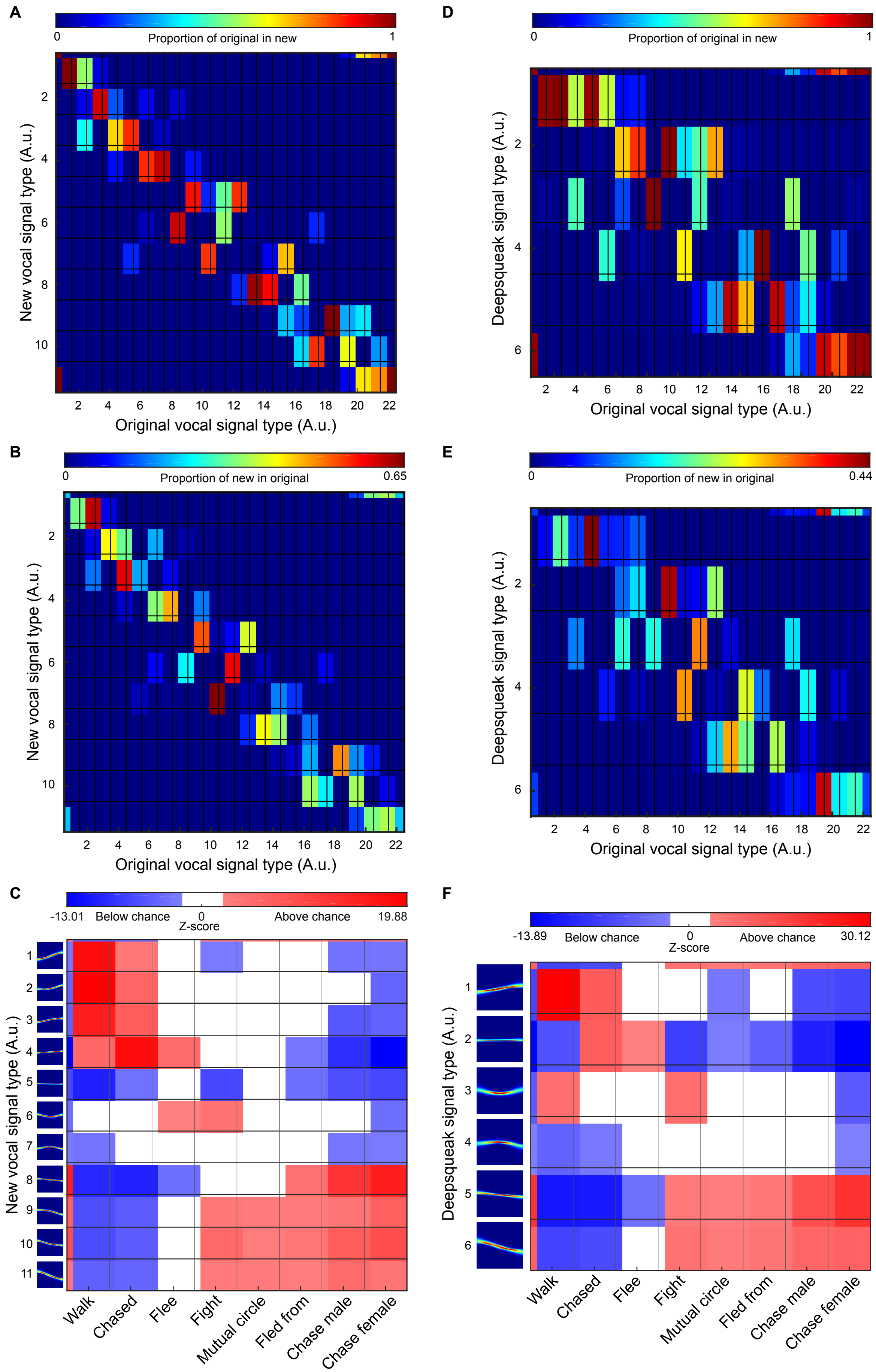
Supplementary Fig. 18. Examples of social context-dependent vocal expression for all males. Randomly selected examples showing the types of vocal signals emitted in specific behavioral contexts by each individual. The patterns of behaviorally-dependent vocal expression for each individual were similar suggesting the patterns of behaviorally-dependent vocal expression are consistent across individuals. Instances of each behavior are shown in white and indicated on the x-axis. A maximum of 15 random instances of a behavior containing a vocal signal and lasting less than 2.5 seconds were selected from each mouse for visualization purposes. The mouse of interest is shown in black using a binary index below each example. If there were fewer than 15 instances of a behavior, then every example was shown. Vocal signal types are indicated by uniquely colored rectangles. Spectrograms show the vocal signal type**.**

Supplementary Figure 19



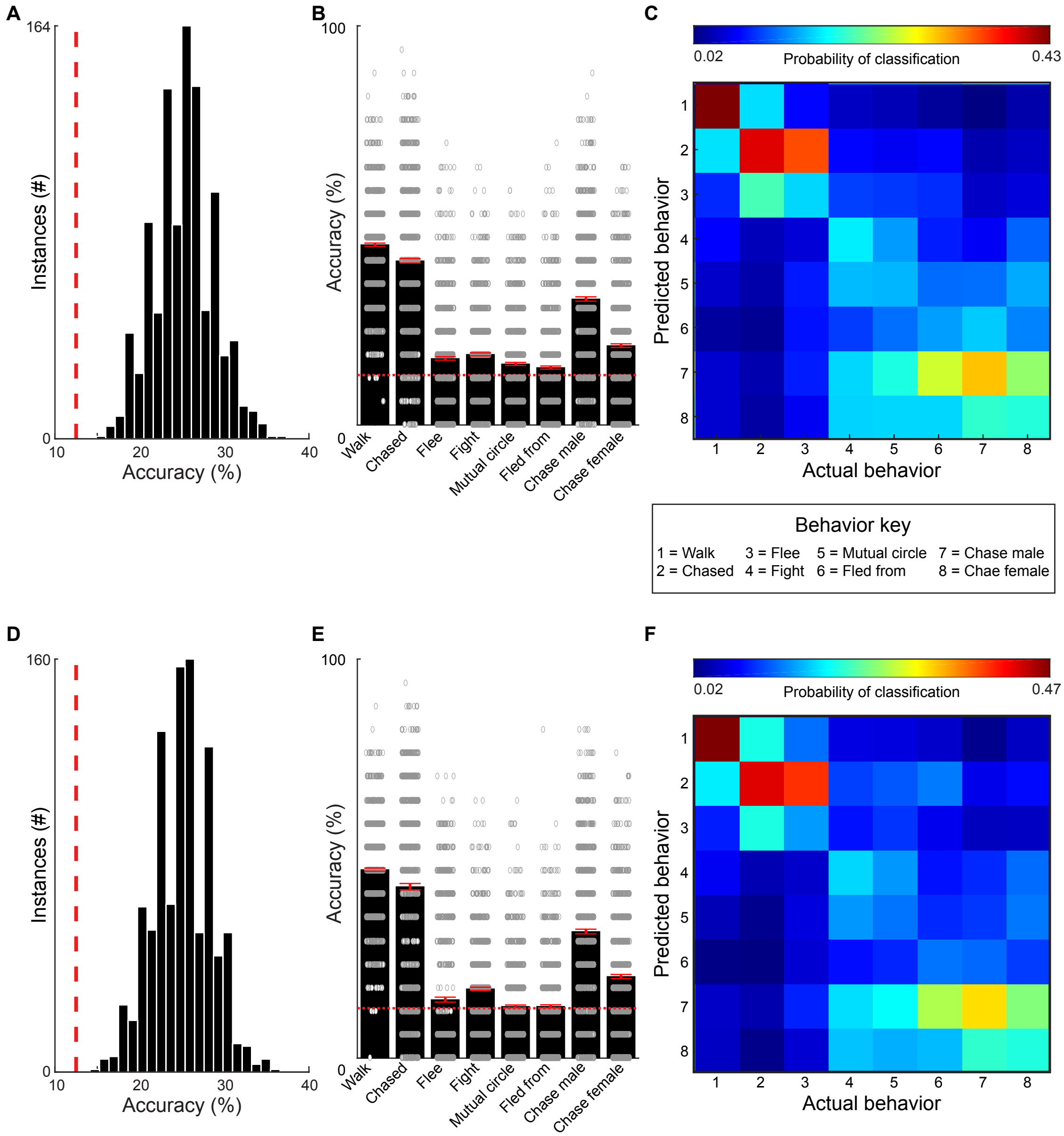
Supplementary Fig. 19. Behaviorally-dependent vocal emission across individuals. A, Proportion of each vocal signal type during each behavior. Proportion was calculated for each individual and then collapsed across individuals. Red line = average, width of line = ±SEM; n=3,586 biologically independent samples analyzed, 2-way ANOVA, F=30.66, p<10-25. **B, C,** Examples of vocal signal emission when the same mouse is chasing versus being chased. Timeline shows the position and type of signals emitted by mice during the initial, middle, and final stages of the behavior. Vocalizing mouse indicated by red outline. Signal type and duration indicated in bottom left and top right of spectrogram, respectively. Similar results were repeated independently in all 11 experiments. **D,** Vocal index indicating proportion of signal types emitted as a male was chasing another male or being chased. Values closer to 1 indicate increased vocal emission when males were chasing another male and values closer to -1 indicate increased vocal emission when males were being chased. Bar graph below indicates how many mice contributed examples to the analysis. Red line = average, width of line = ±SEM; n=376 biologically independent samples analyzed, 1-way ANOVA, F=6.8, p<10-15 **E,** 2-sided Fisher's least significant difference was used for multiple comparisons and multiple comparisons were accounted for using the Benjamini-Hochberg procedure (red and grey, significant and non-significant, respectively).

Supplementary Figure 20



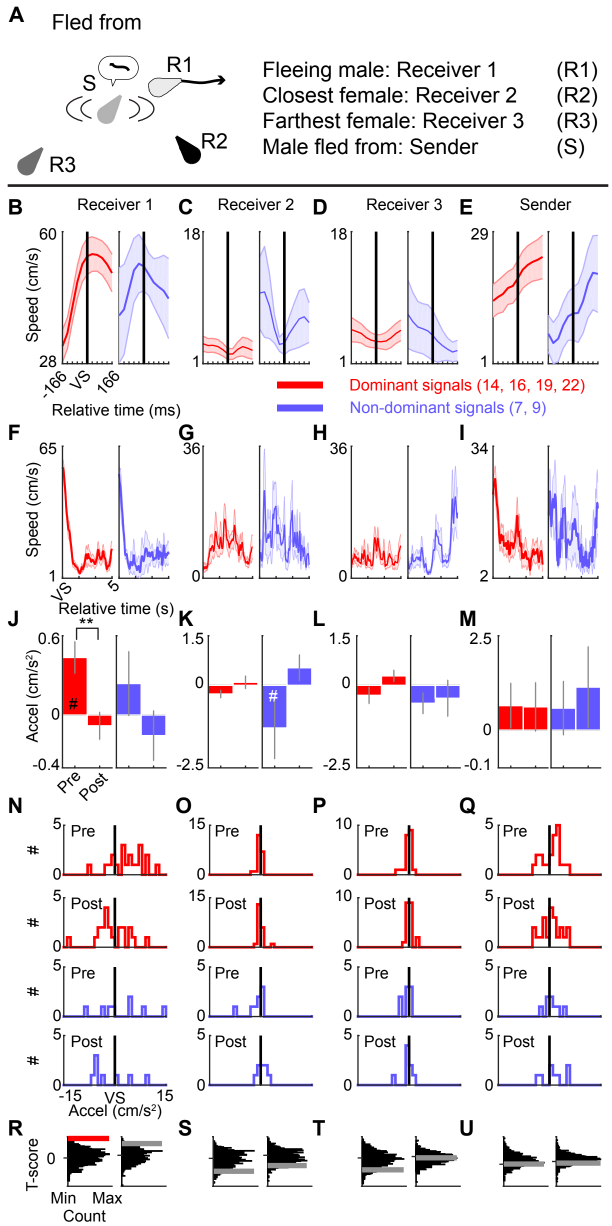
Supplementary Fig. 20. Behaviorally-dependent vocal expression using a reduced number of signal types. Two approaches were used to reduce the similarity between vocal signal types. **A-C,** The total number of signal types was reduced by 50 percent. **D-F,** Vocal signals were categorized using a deep learning approach, “DeepSqueak”. **A,D,** Composition of the 11 or 6 new vocal signal types. Proportion shows the number of originally clustered signals in the new signal type. **B,E,** The proportion of originally clustered vocal signal types that went into each of the 11 or 6 new vocal signal types. **C,F,** Behaviorally-dependent vocal signaling. Different types of signals were emitted above (red), below (blue), and at chance (white) in different behaviors. Importantly, the results are similar to **Fig. 6A**. 2-sided permutation tests, n=1000 independent permutations for each analysis.

Supplementary Figure 21



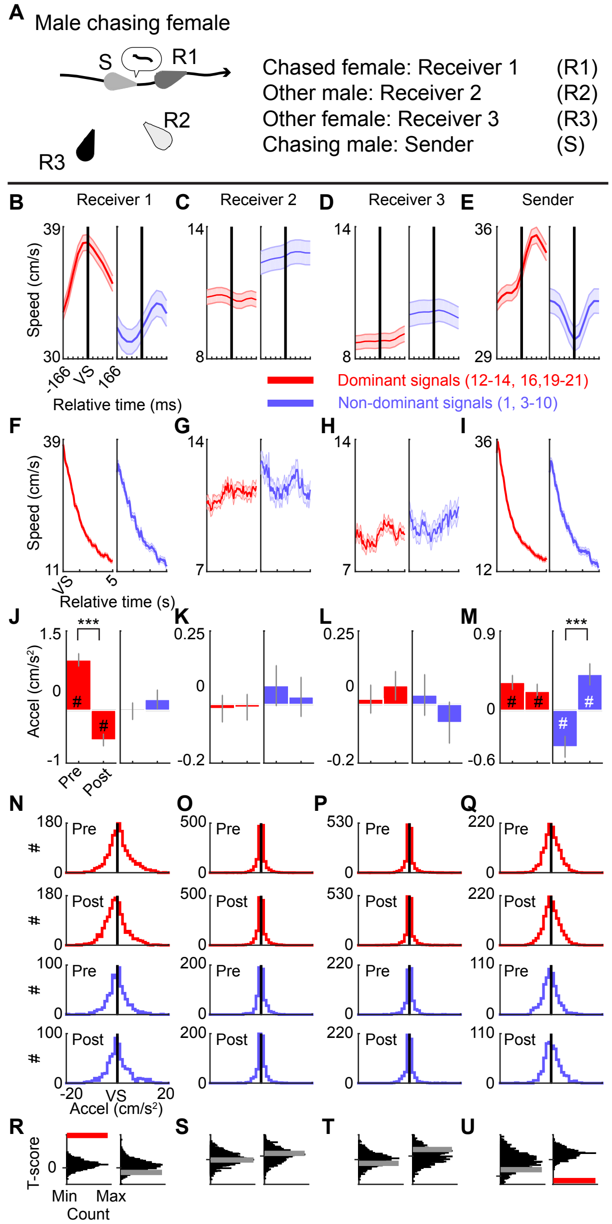
Supplementary Fig. 21. Decoding behavior based on a reduced number of vocal signal types. Two approaches were used to reduce the similarity between vocal signal types. **A-C,** The total number of signal types was reduced by 50 percent. **D-F,** Vocal signals were categorized using a deep learning approach, "DeepSqueak". **A,D,** Distributions of classifier accuracies (11 clusters, 1-sided permutation test, n=1000 permutations, z=-3.57, $p<10^{-4}$; 6 clusters, 1-sided permutation test, n=1000 permutations, z-3.57, $p<10^{-4}$). **B,E,** Classifier accuracy for each behavior (Red line = average, width of line = ±SEM). **C,F,** Confusion matrices showing classification errors.

Supplementary Figure 22



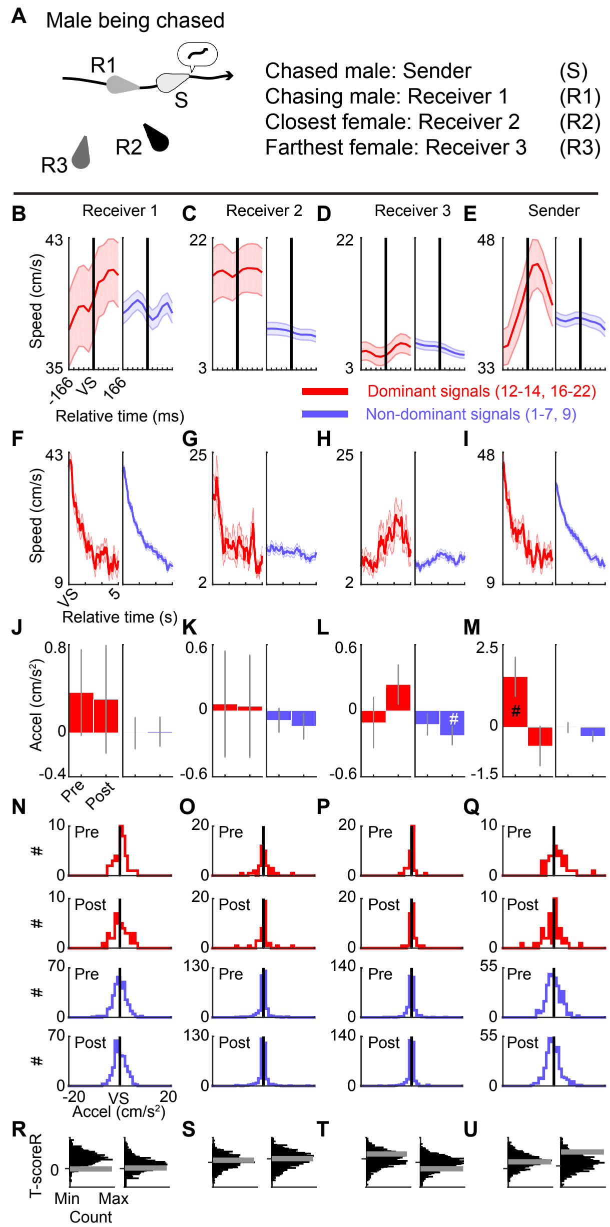
Supplementary Fig. 22. Behavioral changes associated with vocal signals emitted by a male being fled from. A, Schematic of a male mouse (Sender) being fled from by another male (Receiver 1) as well as the closest (Receiver 2) and farthest (Receiver 3) females. **B-E,** Average speed±SEM for Receiver 1 (**B**), Receiver 2 (**C**), Receiver 3 (**D**), and Sender (**E**) before and after the emission of dominant (red) or non-dominant (blue) vocal signals (VS). There were 21 dominant and 8 non-dominant vocal signals included in the analyses. **F-I,** Average speed±SEM in the 5 seconds after the emission of dominant or non-dominant vocal signals. **J-M,** Acceleration of animals (Average±SEM; # indicates that an animal was accelerating (2-sided 1-sample t-test, T=3.51 p=0.002) or decelerating (2-sided 1-sample t-test, significant T=-2.19, significant p=0.04; ** denotes significant differences in pre- and post-acceleration, 2-sided paired t-test, T=2.75, p=0.01). **N-Q,** Full data distribution for **J-M**. **R-U,** Randomly generated distributions of T-scores comparing pre- and post-acceleration calculated for signals that were randomly shifted to different times within a flee. T-score distributions were compared to the T-scores calculated in **J-M**. Red horizontal bar in **R** indicates above chance, 2-sided permutation test, n=1000 independent permutations, p<0.05; gray horizontal bars in **R-U** indicate chance, 2-sided permutation tests, n=1000 independent permutations, all p>0.07. Panels: left and right, dominant and non-dominant signals, respectively.

Supplementary Figure 23



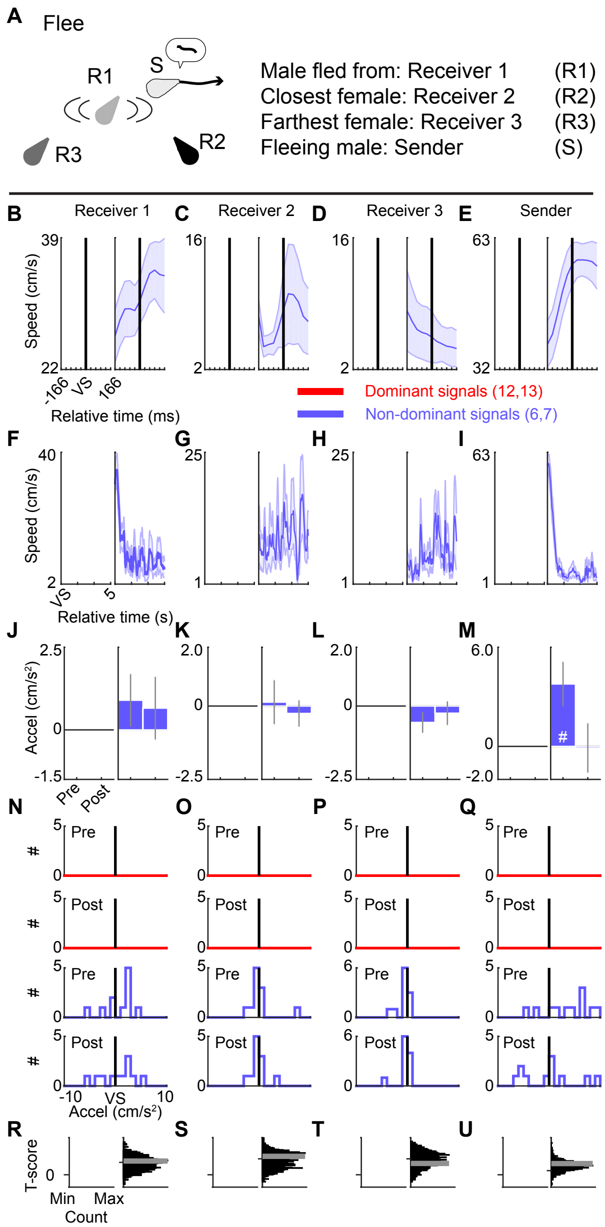
Supplementary Fig. 23. Behavioral changes associated with vocal signals emitted by a male chasing a female. A, Schematic of a male mouse (Sender) chasing a female (Receiver 1) as well as the other non-engaged male (Receiver 2) and female (Receiver 3). **B-E,** Average speed±SEM for Receiver 1 (**B**), Receiver 2 (**C**), Receiver 3 (**D**), and Sender (**E**) before and after the emission of dominant (red) or non-dominant (blue) vocal signals (VS). There were 1424 dominant and 616 non-dominant vocals signals included in the analyses. **F-I,** Average speed±SEM in the 5 seconds after the emission of dominant or non-dominant vocal signals. **J-M,** Acceleration of animals (Average±SEM; # indicates that an animal was accelerating (2-sided 1-sample t-test, all significant $T>2.59$, all significant $p<0.01$) or decelerating (2-sided 1-sample t-test, all significant $T<-3.45$, all significant $p<0.01$; *** denotes significant differences in pre- and post-acceleration, 2-sided paired t-test, $T>4.24$ $p<0.001$). **N-Q,** Full data distribution for **J-M**. **R-U,** Randomly generated distributions of T-scores comparing pre- and post-acceleration calculated for signals that were randomly shifted to different times within a chase. T-score distributions were compared to the T-scores calculated in **J-M**. Red horizontal bars in **R** and **U** indicate above and below chance, 2-sided permutation test, n=1000 independent permutations, all $p<0.05$; gray horizontal bars in **R-U** indicate chance, 2-sided permutation tests, n=1000 independent permutations, all $p>0.17$. Panels: left and right, dominant and non-dominant signals, respectively.

Supplementary Figure 24



Supplementary Fig. 24. No immediate behavioral changes associated with vocal signals emitted by a male being chased by another male. A, Schematic of a male mouse (Sender) being chased by another male (Receiver 1) as well as the closest (Receiver 2) and farthest (Receiver 3) females. **B-E,** Average speed±SEM for Receiver 1 (**B**), Receiver 2 (**C**), Receiver 3 (**D**), and Sender (**E**) before and after the emission of dominant (red) or non-dominant (blue) vocal signals (VS). There were 40 dominant and 318 non-dominant vocals signals included in the analyses. **F-I,** Average speed±SEM in the 5 seconds after the emission of dominant or non-dominant vocal signals. **J-M,** Acceleration of animals (Average±SEM; # indicates that an animal was accelerating (2-sided 1-sample t-test, significant T=2.54, significant $p<0.05$) or decelerating (2-sided 1-sample t-test, significant T<-2.59, significant $p<0.05$). **N-Q,** Full data distribution for **J-M**. **R-U,** Randomly generated distributions of T-scores comparing pre- and post-acceleration calculated for signals that were randomly shifted to different times within a chase. T-score distributions were compared to the T-scores calculated in **J-M**. Gray horizontal bars in **R-U** indicate chance, 2-sided permutation tests, n=1000 independent permutations, all $p>0.06$. Panels: left and right, dominant and non-dominant signals, respectively.

Supplementary Figure 25



Supplementary Fig. 25. No immediate behavioral changes associated with vocal signals emitted by a male fleeing another male. A, Schematic of a male mouse (Sender) fleeing from another male (Receiver 1) as well as the closest (Receiver 2) and farthest (Receiver 3) females. **B-E,** Average speed±SEM for Receiver 1 (**B**), Receiver 2 (**C**), Receiver 3 (**D**), and Sender (**E**) before and after the emission of dominant (red) or non-dominant (blue) vocal signals (VS). Note, no dominant vocal signals emitted by the fleeing animal were recorded in isolation (i.e., no other vocal signals within ±166 ms). There were 0 dominant and 11 non-dominant vocals signals included in the analyses. **F-I,** Average speed±SEM in the 5 seconds after the emission of dominant or non-dominant vocal signals. **J-M,** Acceleration of animals (Average±SEM; # indicates that an animal was accelerating, 2-sided 1-sample t-test, significant T=2.72, significant p=0.02). **N-Q,** Full data distribution for **J-M**. **R-U,** Randomly generated distributions of T-scores comparing pre- and post-acceleration calculated for signals that were randomly shifted to different times within a flee. T-score distributions were compared to the T-scores calculated in **J-M**. Gray horizontal bars in **R-U** indicate chance, 2-sided permutation tests, n=1000 independent permutations, all p>0.60. Panels: left and right, dominant and non-dominant signals, respectively.