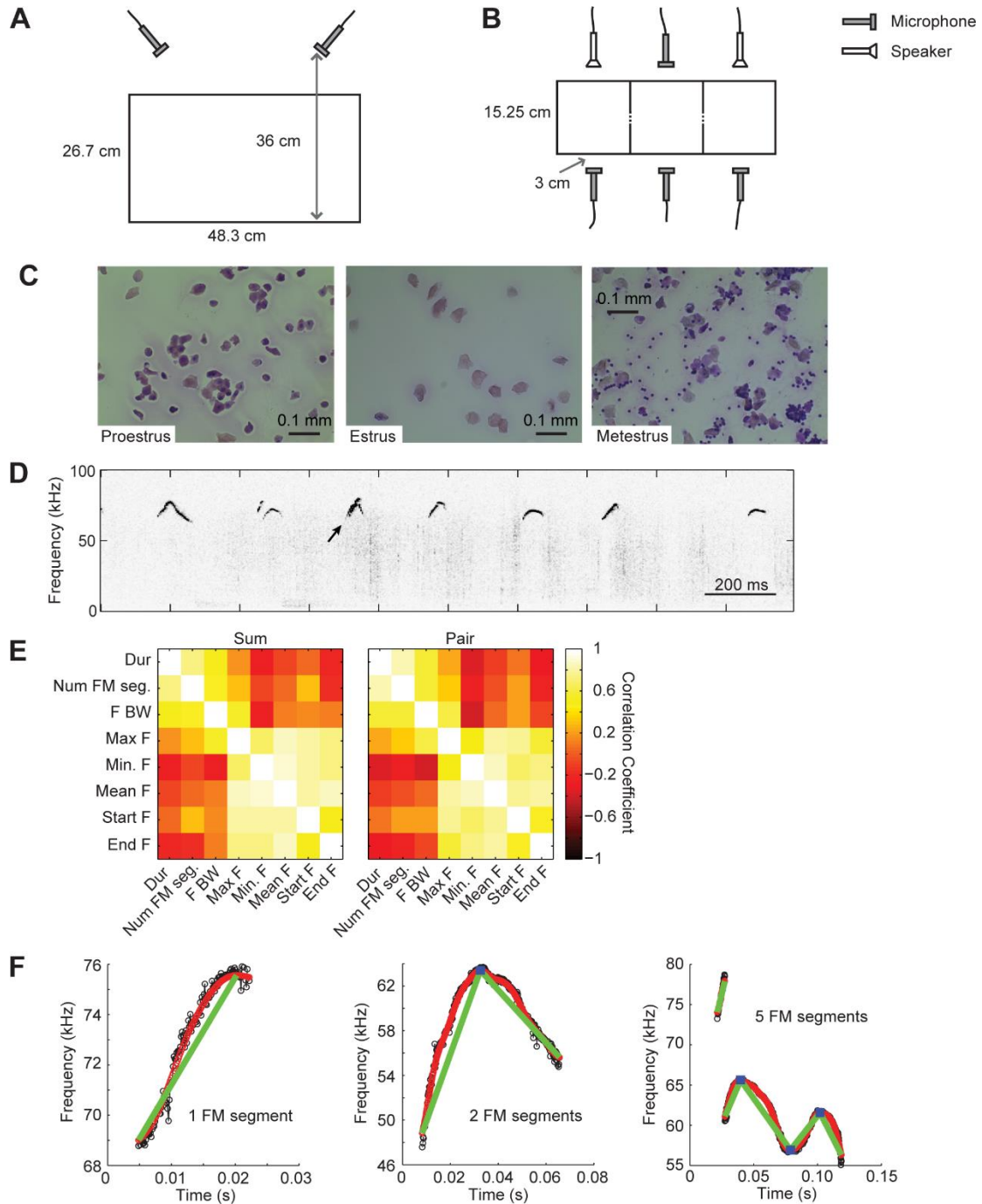
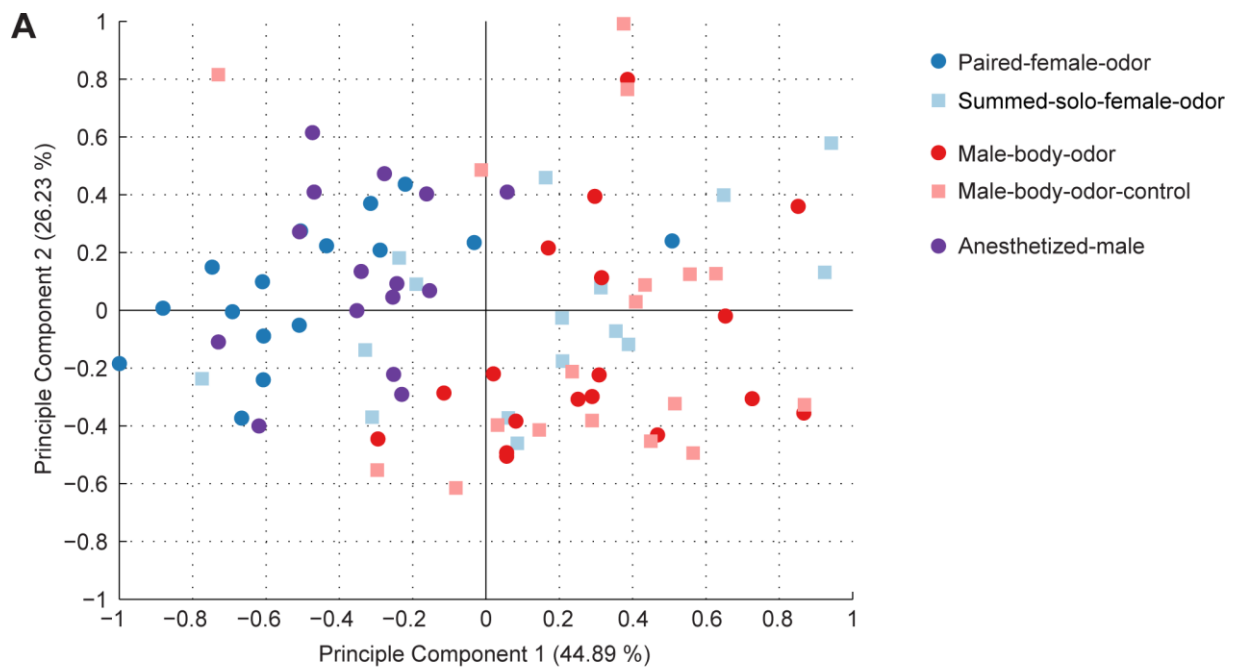


SUPPLEMENTAL FIGURES



Supplemental Figure 1. Methods. (A) Schematic side view of arena used in Experiments 1-4 and 6. (B) Schematic top view of arena used in Experiment 5. (C) Example slides of vaginal cells collected in proestrus, estrus, and metestrus. Proestrus was characterized as a mixture of basal and squamous cells, with few neutrophils, and estrus was characterized as a large

proportion of squamous cells, with few basal cells or neutrophils. Metestrus and diestrus were combined together due to few observations of diestrus and were characterized as a mixture of basal cells, squamous cells, and neutrophils. (D) Example of overlapping vocalizations from two mice (see arrow). (E) Correlations between measured characteristics of acoustic structure in the Summed-solo-female-odor (Sum) and Paired-female-odor (Pair) conditions (Dur = duration, F = frequency, BW = bandwidth, FM seg. = frequency-modulated segments). (F) Frequency-modulated (FM) segment examples. Black circles are original data points from the frequency contour, and red circles are the interpolated and smoothed contour. Green lines show the frequency modulation of frequency-modulated segments and blue markers show where the frequency modulation changed sign.



B

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7
% Variance explained	44.89%	26.23%	13.14%	8.74%	3.80%	3.14%	0.06%
Duration	-0.50	-0.16	-0.06	-0.11	0.41	0.74	0.01
Frequency BW	0.15	-0.65	0.14	0.14	0.63	-0.36	0.04
Mean Frequency	0.24	0.06	0.91	-0.26	-0.02	0.22	-0.01
Complex	-0.51	-0.18	0.13	-0.32	-0.23	-0.31	0.66
Upsweeps	0.52	-0.17	-0.15	0.26	-0.14	0.41	0.66
Downsweeps	-0.37	0.12	0.34	0.85	-0.08	-0.02	0.05
Flats	0.06	0.69	-0.02	-0.03	0.60	-0.15	0.36

Supplemental Figure 2. Principle component analysis of acoustic structure features, complexity, and simple syllable types. Duration and downsweep data were log transformed to make them more normally distributed (a constant of 0.0001 to each downsweep data point to avoid zero values). (A) Principle components 1 and 2 plotted against one another. (B) Component loadings and % variance explained for all seven principle components. Duration = vocalization duration at the 92nd percentile. Frequency BW = frequency bandwidth at the 50th percentile. Mean Frequency = vocalization mean frequency at the 50th percentile. Complex = proportion complex vocalizations. Upsweeps = proportion upsweep vocalizations. Downsweeps = proportion downsweep vocalizations. Flats = proportion flat vocalizations.

Supplemental Table 1. Results of a generalized linear mixed model examining the effects of odor condition and social context on USV count. The response variable was the vocalization counts from the Summed-solo-no-odor, Paired-no-odor, Summed-solo-female-odor, and Paired-female-odor conditions (USV_count), while Odor_condition (no-odor versus female-odor) and Social_context (Summed-solo versus Paired) were fixed factors. Subject ID (n = 20 subjects) was coded as a random factor. The formula given is the model specification formula written in Wilkinson's notation. The comparison groups are also written using Wilkinson's notation.

Formula: USV_count ~ 1 + Odor_condition*Social_context + (1 | Subject1) + (1 | Subject2) + ... + (1 | Subject20)

Comparison groups	Statistical test	α	Test statistic	p-value
Odor_condition	F-test	0.05	F = 3698.6	1.227×10^{-63}
Social_context	F-test	0.05	F = 277.85	2.015×10^{-26}
Odor_condition:Social_context	F-test	0.05	F = 17.27	8.817×10^{-5}

Supplemental Table 2. Results of a generalized linear mixed model investigating the effect of social context on principle component 1 from the acoustic structure PCA. Social context was a fixed factor, Subject ID was a random factor, and Principle Component 1 was the response variable. All five social contexts from the principle component analysis were included (52 unique subjects). The formula given is the model specification formula written in Wilkinson's notation.

Formula: PrincipleComponent1 ~ 1 + Social_context + (1 | Subject1) + (1 | Subject2) + ... + (1 | Subject52)

Comparison groups	Statistical test	α	Test statistic	p -value
Social_context	F -test	0.05	$F = 26.79$	5.391×10^{-14}
Paired-female-odor vs. Summed-solo-female-odor	F -test	0.05	$F = 51.69$	3.427×10^{-10}
Male-body-odor vs. Male-body-odor-control	F -test	0.05	$F = 0.175$	0.677
Anesthetized-male vs. Summed-solo-female-odor	F -test	0.05	$F = 34.94$	8.493×10^{-8}
Anesthetized-male vs. Paired-female-odor	F -test	0.05	$F = 0.122$	0.728

Supplemental Table 3. Results of a generalized linear mixed model investigating the effect of social context on principle component 2 from the acoustic structure PCA. Social context was a fixed factor, Subject ID was a random factor, and Principle Component 2 was the response variable. All five social contexts from the principle component analysis were included (52 unique subjects). The formula given is the model specification formula written in Wilkinson's notation.

Formula: PrincipleComponent2 ~ 1 + Social_context + (1 | Subject1) + (1 | Subject2) + ... + (1 | Subject52)

Comparison groups	Statistical test	α	Test statistic	p -value
Social_context	F -test	0.05	$F = 13.00$	3.686×10^{-8}
Paired-female-odor vs. Summed-solo-female-odor	F -test	0.05	$F = 1.127$	0.292
Male-body-odor vs. Male-body-odor-control	F -test	0.05	$F = 1.489$	0.226
Anesthetized-male vs. Summed-solo-female-odor	F -test	0.05	$F = 2.417$	0.124
Anesthetized-male vs. Paired-female-odor	F -test	0.05	$F = 0.386$	0.536