
Figures and figure supplements

A fear conditioned cue orchestrates a suite of behaviors in rats

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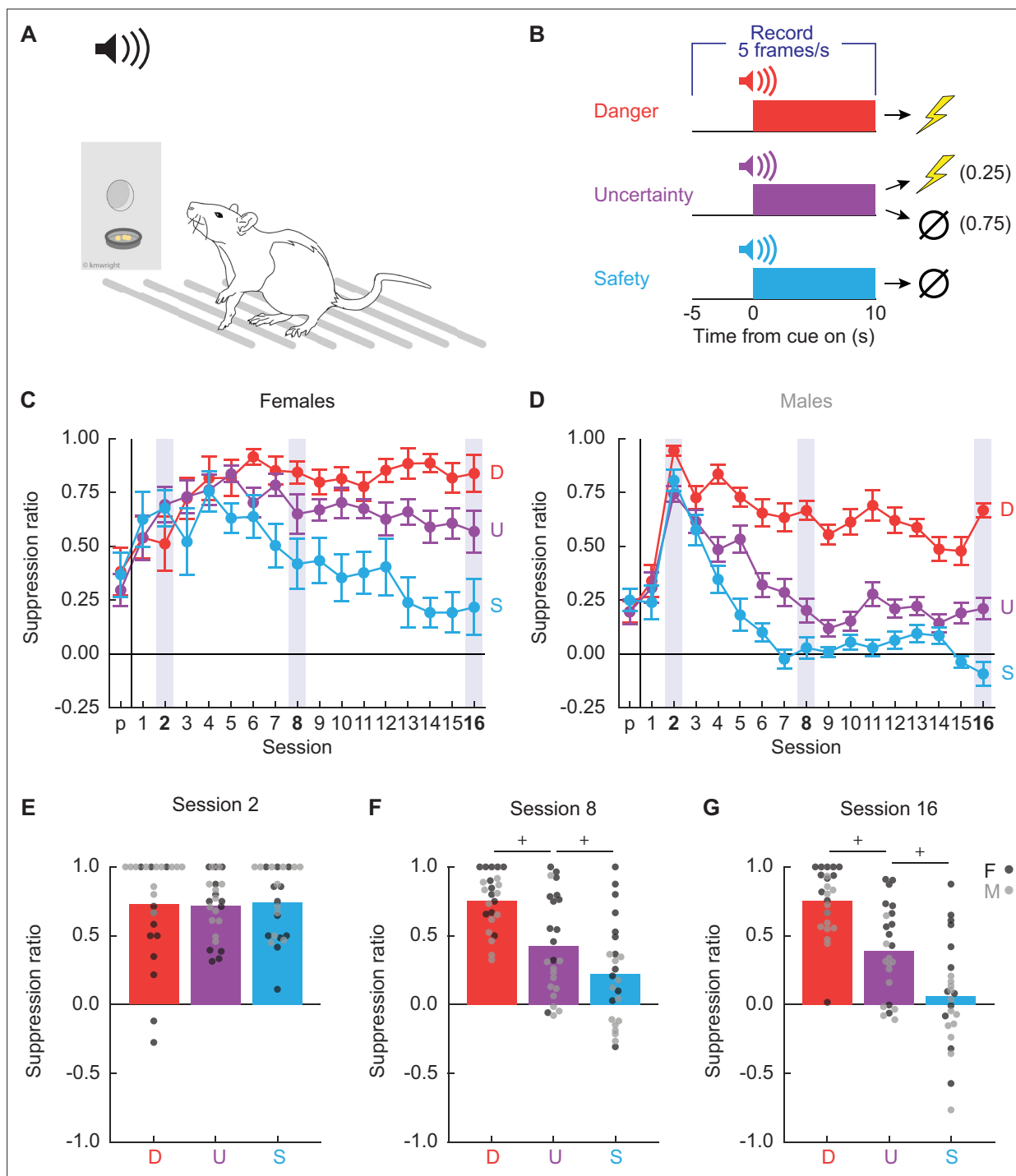


Figure 1. Experimental design and nose poke suppression. **(A)** Conditioned suppression procedure during which rats nose poke for food, while cues are played overhead and shocks delivered through floor. **(B)** Fear discrimination consisted of 10 s auditory cues predicting unique foot shock probabilities: danger (red; $p = 1$), uncertainty (purple; $p = 0.25$), and safety (blue; $p = 0$). Five video frames were captured per second, starting 5 s prior to cue onset and continuing through cue presentation. Mean \pm standard error of the mean (SEM) suppression ratios for danger (red), uncertainty (purple), and safety (blue) from pre-exposure through discrimination session 16 are shown for **(C)** females and **(D)** males. Mean \pm individual suppression ratios for each cue are shown for **(E)** session 2, **(F)** session 8, and **(G)** session 16. Individuals represented by black (female) and gray (male) dots. +95% bootstrap confidence interval does not contain zero.

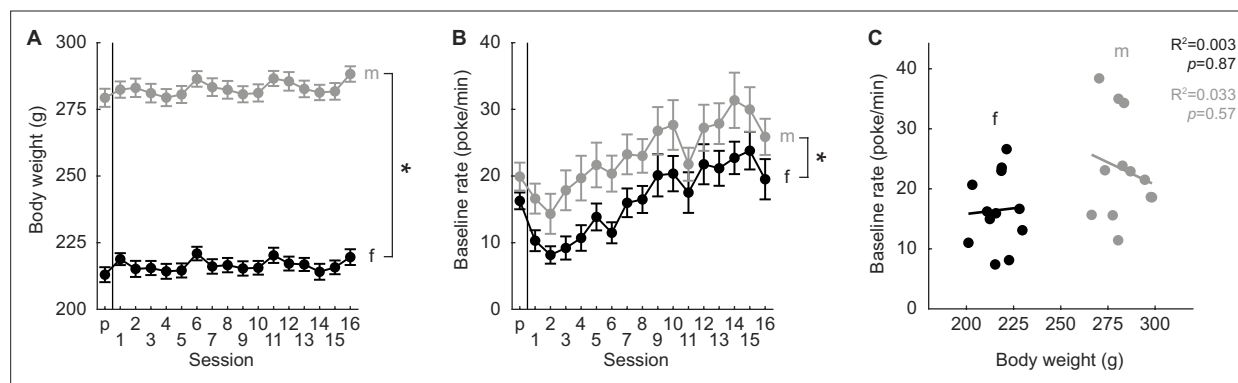


Figure 1—figure supplement 1. Body weight and baseline nose poke rate. **(A)** Analysis of variance (ANOVA) for body weight (g) [factors: sex and session] revealed significant main effects of session ($F_{(16,352)} = 29.58$, $p = 1.90 \times 10^{-55}$), sex ($F_{(1,22)} = 287.54$, $p = 4.07 \times 10^{-14}$), and a significant session \times sex interaction ($F_{(16,352)} = 2.20$, $p = 0.005$). Mean \pm standard error of the mean (SEM) body weights in grams (y-axis) of males (gray) and females (black) from pre-exposure through session 16. **(B)** Baseline nose poke rates (poke/min) decreased during discrimination sessions 1 and 2, then increased over the remaining sessions. Males poked at higher baseline levels across all sessions. ANOVA for baseline nose poke rate (poke/min) [factors: sex and session] revealed significant main effects of session ($F_{(16,352)} = 19.30$, $p = 4.44 \times 10^{-39}$) and sex ($F_{(1,22)} = 5.10$, $p = 0.034$). Mean baseline pose rate (y-axis) of males and females from sessions 1 to 16. **(C)** Baseline nose poke rate plotted against body weight for all individuals. There was no relationship between the two measures in either female or male rats. *Paired samples t-test $p < 0.05$.

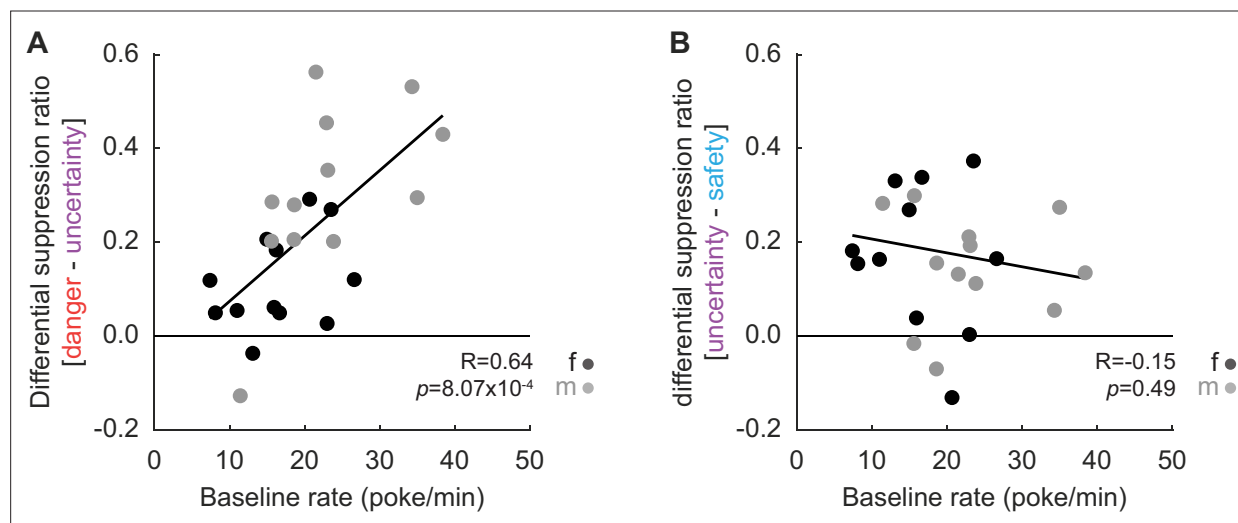


Figure 1—figure supplement 2. Nose poke \times discrimination. Correlations between baseline nose poke rate and differential suppression ratios for (A) danger and uncertainty, and (B) uncertainty and safety are shown. Individuals represented by black (female) and gray (male) circles. R and p values from Pearson's correlation coefficient reported.

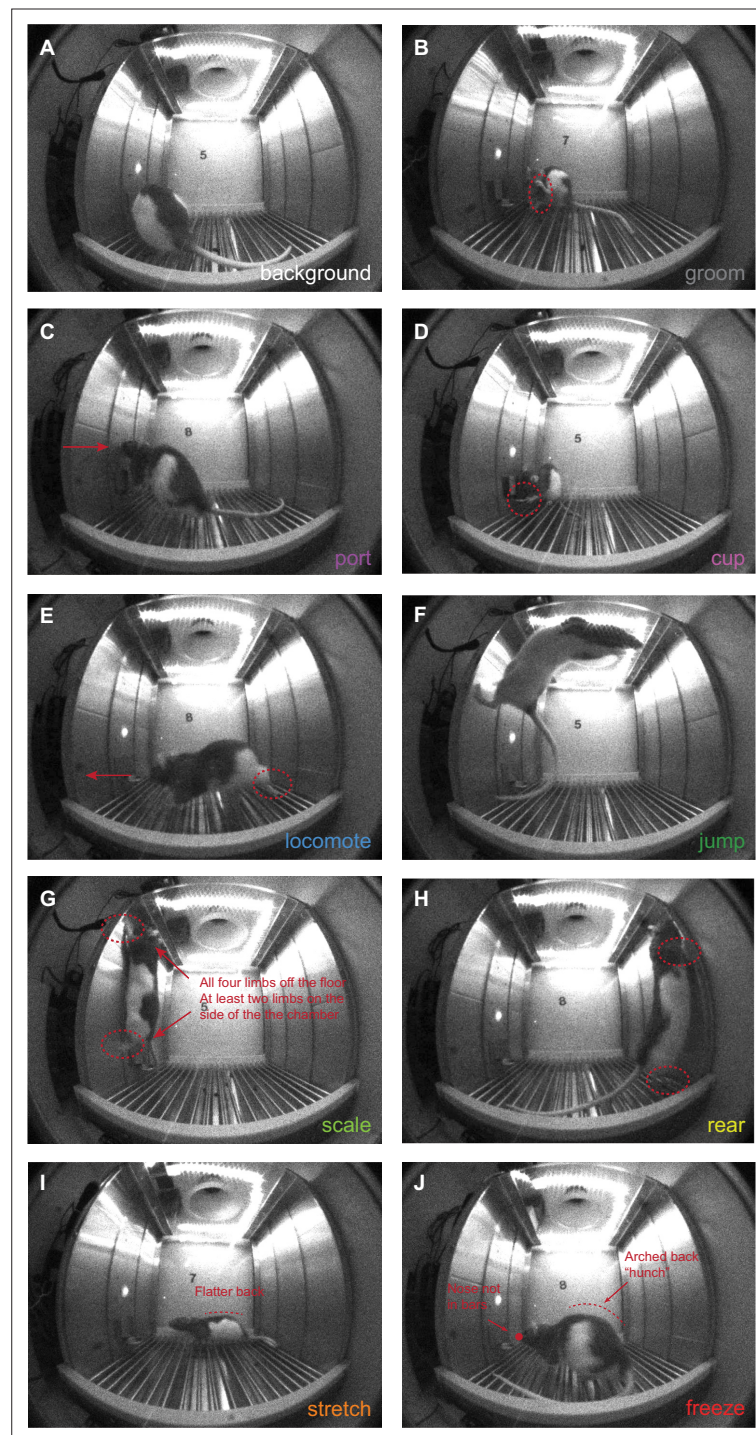


Figure 2. Representative behaviors. Representative frames are shown for: (A) background, (B) groom, (C) port, (D) cup, (E) locomote, (F) jump, (G) scale, (H) rear, (I) stretch, and (J) freeze.

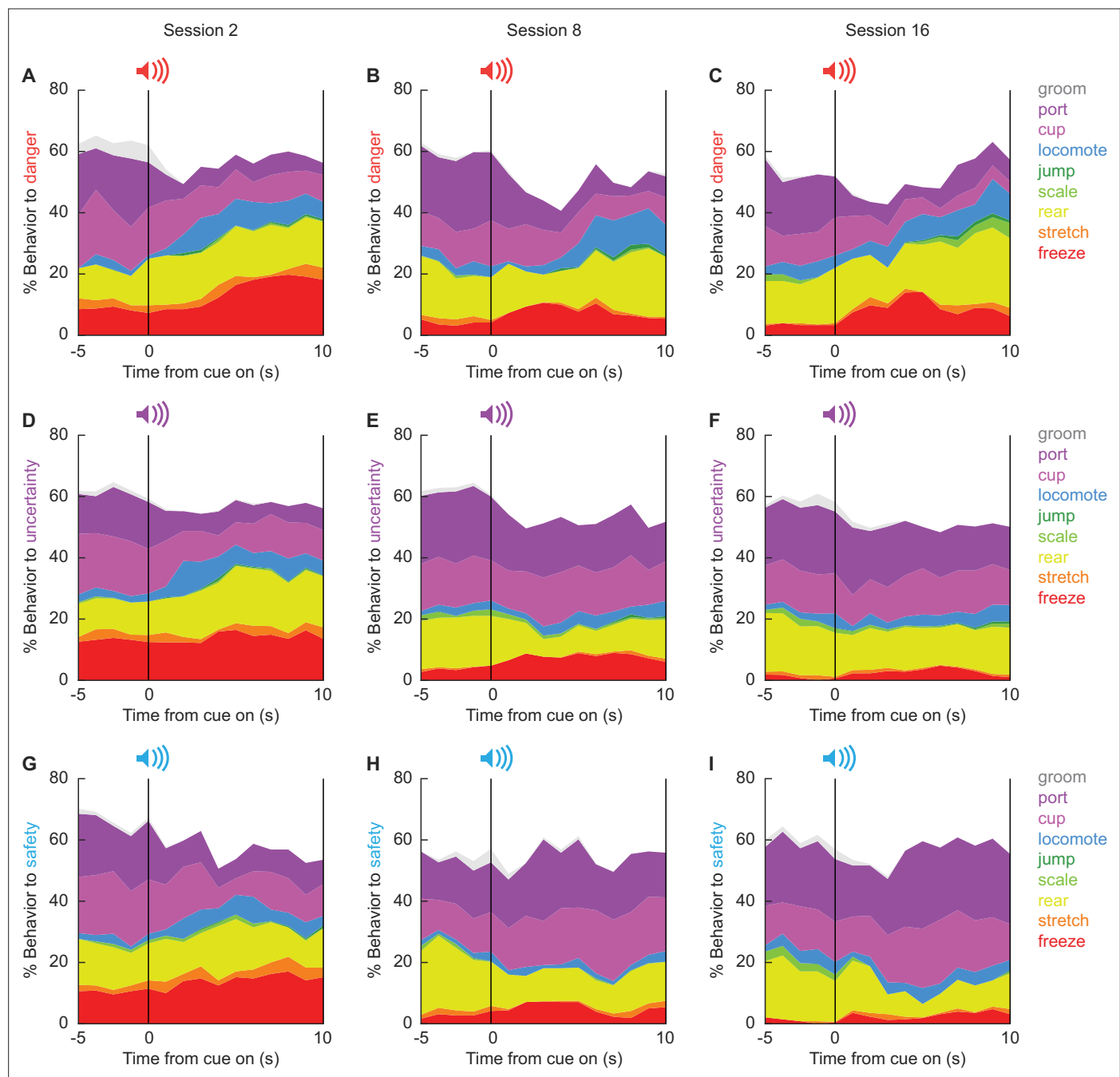


Figure 3. Temporal ethograms. Mean percent behavior from 5 s prior through 10 s cue presentation is shown for the danger cue during sessions (A) 2, (B) 8, and (C) 16; the uncertainty cue during sessions (D) 2, (E) 8, and (F) 16; and the safety cue during sessions (G) 2, (H) 8, and (I) 16. Behaviors are groom (gray), port (dark purple), cup (light purple), locomote (blue), jump (dark green), scale (light green), rear (yellow), stretch (orange), and freeze (red).

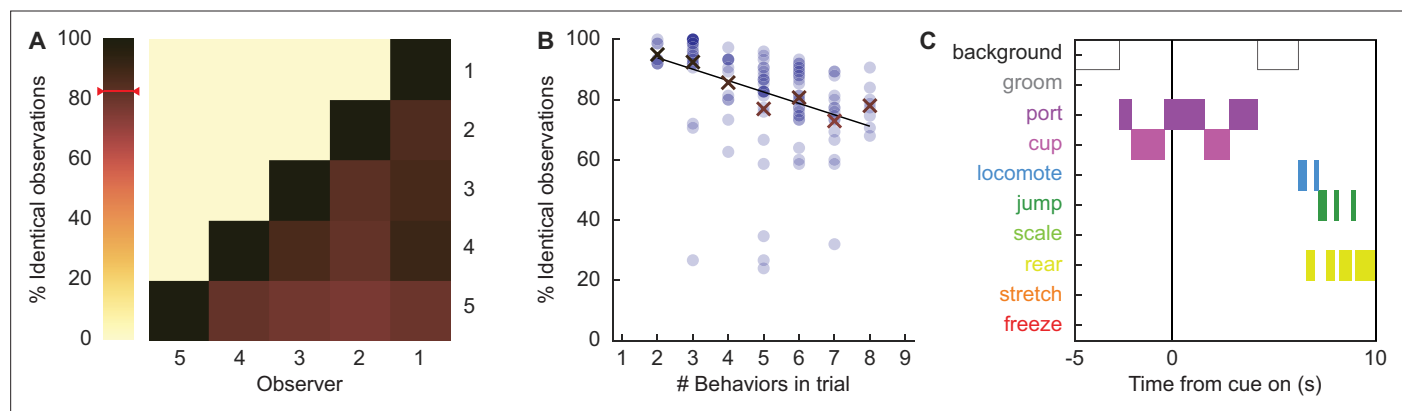


Figure 3—figure supplement 1. Inter-rater reliability. **(A)** Percentage of identical observations between observer–observer pairs. **(B)** Percentage of identical observations as a function of the number of behaviors present in a trial. **(C)** Example ethogram from a single uncertainty cue presentation, taken from a female during session 8. Frames were systematically hand scored by five observers blind to rat identity, session number, and trial type (see Materials and methods for hand scoring approach and trial anonymization). A comparison dataset consisting of 12 trials (900 frames) was also scored by each observer. A correlation matrix compared % identical observations for the 900 comparison frames for each observer–observer pair. Mean % identical observation was 82.83%, with a minimum observer–observer pair agreement of 75.89% and a maximum of 90.56%. Previous studies scoring the presence or absence of freezing have reported inter-observer reliability as an *R* value: 0.93 (Parnas et al., 2005), 0.96 (Pickens et al., 2010), and 0.97 (Jones and Monfils, 2016). Another study simply reported >95% inter-observer agreement (Badrinarayan et al., 2012). These values exceed our mean % identical observation. However, we hand scored nine discrete behaviors. We observed a negative relationship between the number of behavior categories present and % identical observations ($R^2 = 0.17$, $p = 2.27 \times 10^{-6}$). Mean percent identical observation was 95% when two behavior categories were present, and 92.5% when three behavior categories were present. Even when eight behavior categories were present, a mean percent identical observation of 78% was achieved. Our approach yielded high inter-observer reliability across trials with few and many behavior categories present.

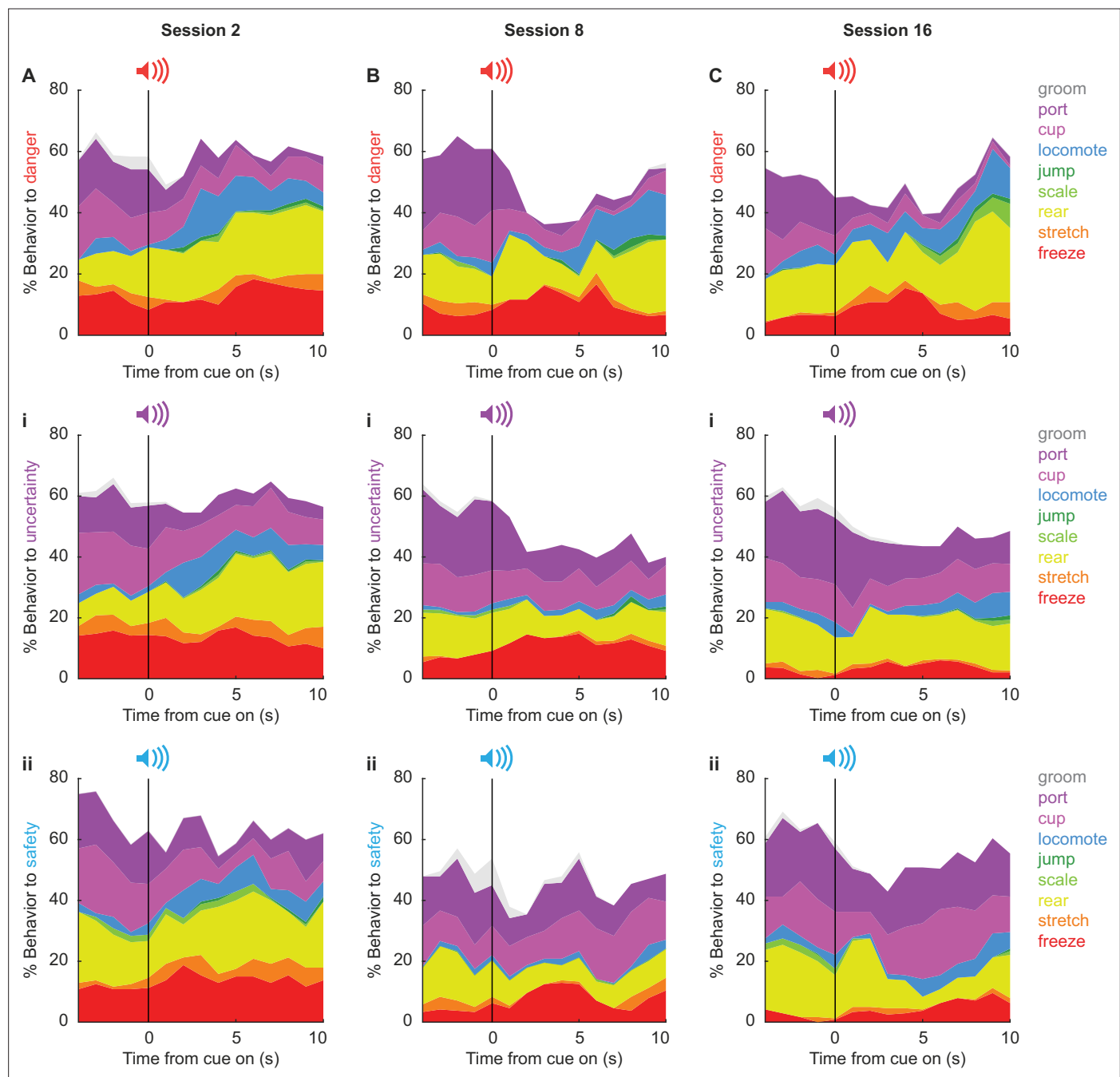


Figure 3—figure supplement 2. Female ethograms. Mean percent behavior from 5 s prior through 10 s cue presentation is shown for danger (row 1), uncertainty (row 2), and safety (row 3) during sessions (A, Ai, Aii) 2, (B, Bi, Bii) 8, and (C, Ci, Cii) 16. Behaviors quantified are groom (gray), port (dark purple), cup (light purple), locomote (blue), jump (dark green), scale (light green), rear (yellow), stretch (orange), and freeze (red).

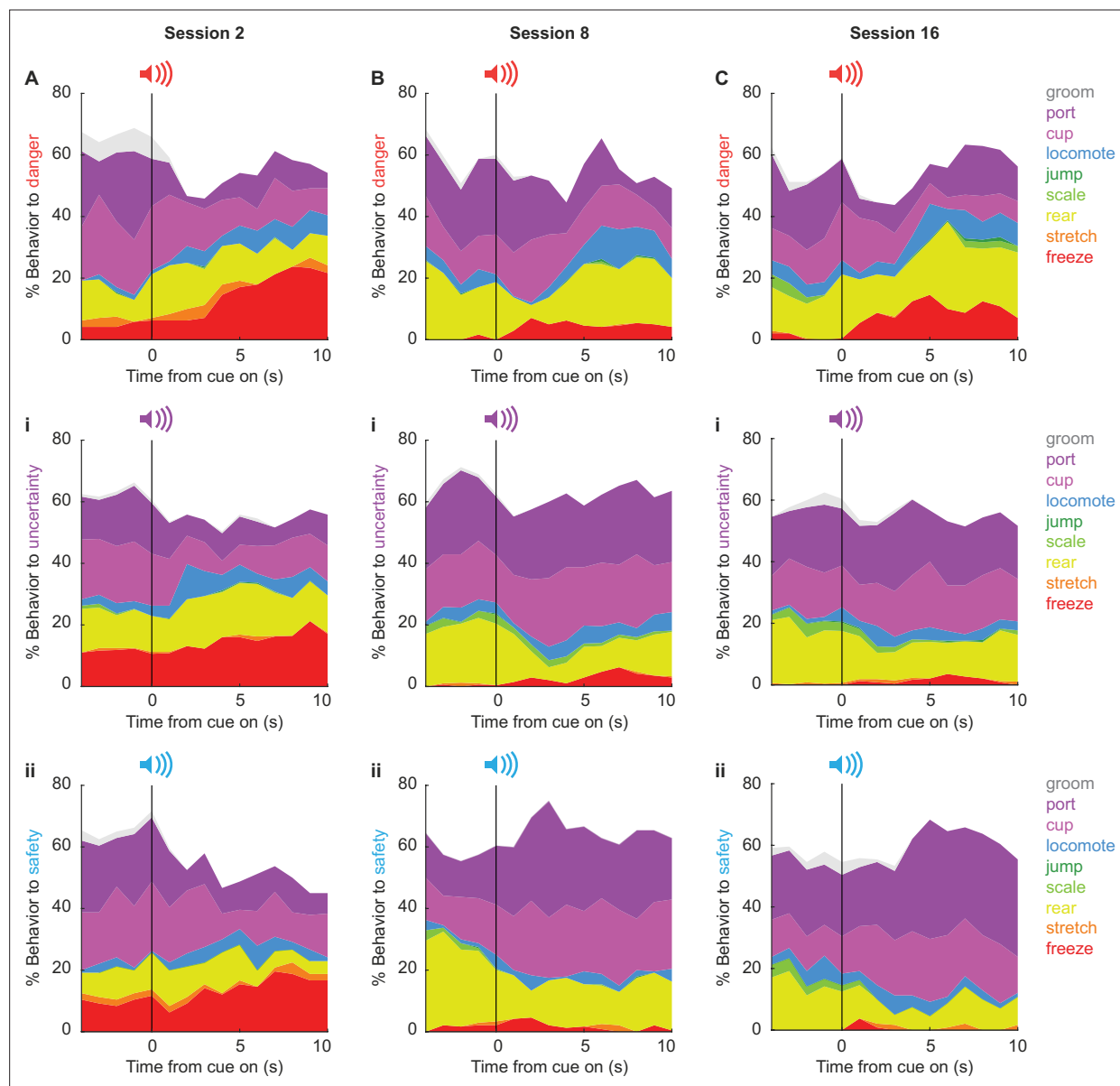


Figure 3—figure supplement 3. Male ethograms. Mean percent behavior from 5 s prior through 10 s cue presentation is shown for danger (row 1), uncertainty (row 2), and safety (row 3) during sessions (A, Ai, Aii) 2, (B, Bi, Bii) 8, and (C, Ci, Cii) 16. Behaviors quantified are groom (gray), port (dark purple), cup (light purple), locomote (blue), jump (dark green), scale (light green), rear (yellow), stretch (orange), and freeze (red).

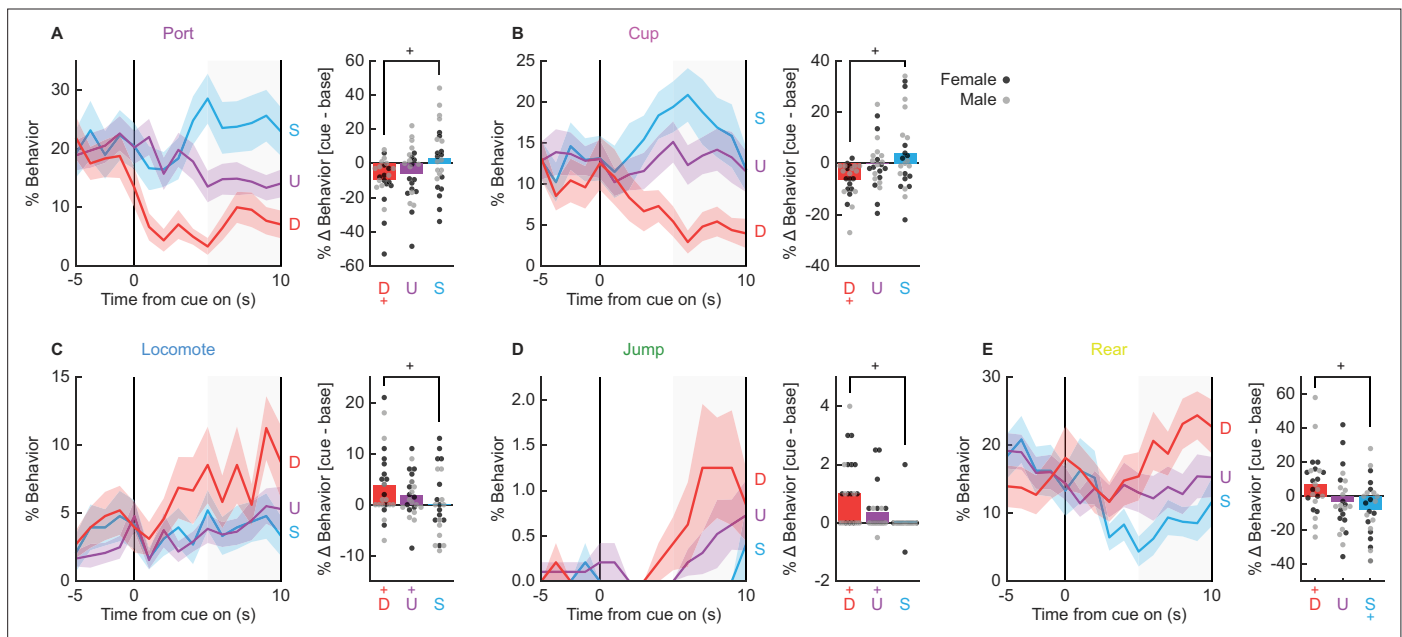


Figure 4. Danger-elicited behaviors. Line graphs show mean \pm standard error of the mean (SEM) percent behavior from 5 s prior through 10 s cue presentation for danger (red), uncertainty (purple), and safety (blue) for (A) port, (B) cup, (C) locomote, (D) jumping, and (E) rearing. Bar plots show mean change in behavior from baseline (5 s prior to cue) compared to last 5 s of cue. Individuals represented by black (female) and gray (male) dots. +95% bootstrap confidence interval for danger vs. safety (black), danger vs. baseline (red), or safety vs. baseline (blue) comparison does not contain zero (black).

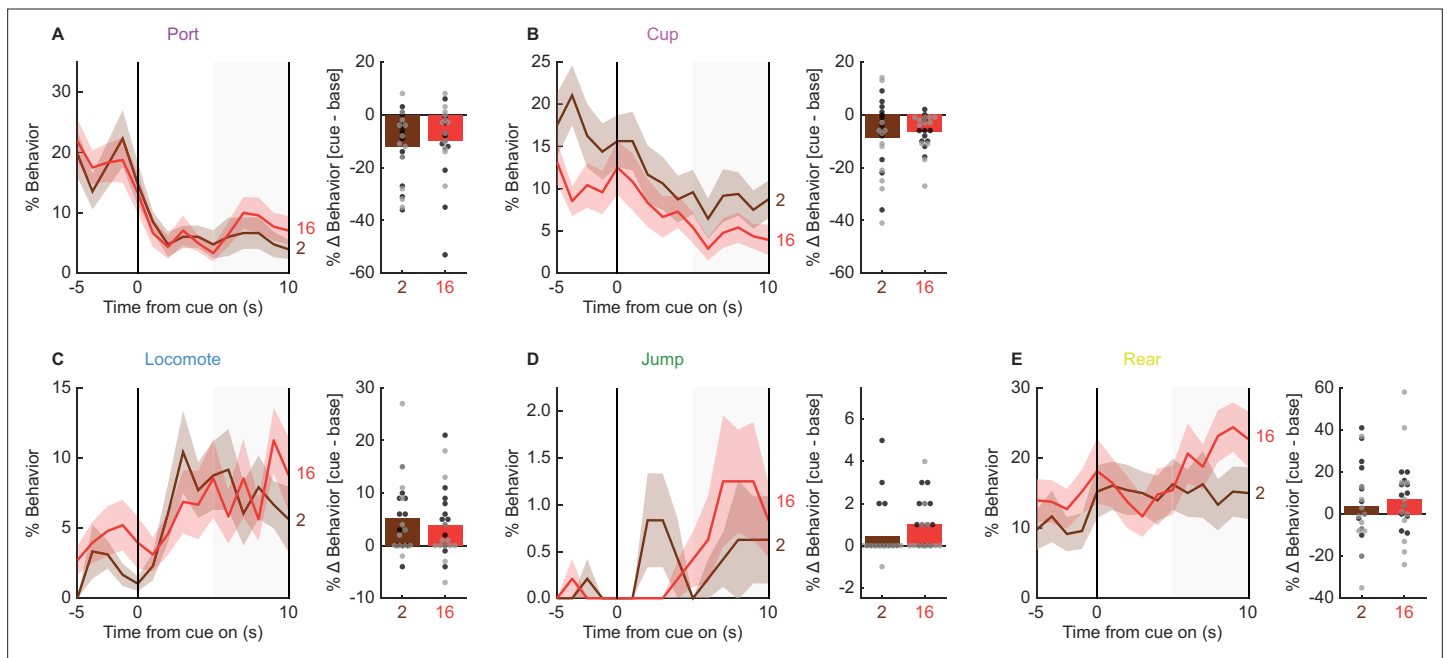


Figure 4—figure supplement 1. Comparison of session 16, danger-specific behaviors during sessions 2 and 16. Mean \pm standard error of the mean (SEM) percent behavior from 5 s prior through 10 s danger presentation is shown for (A) port, (B) cup, (C) locomote, (D) jump, and (E) rear, for sessions 2 (dark brown) and 16 (red). Adjacent plots show mean $\%$ change in behavior from baseline to last 5 s of danger presentation for all rats during sessions 2 (dark brown) and 16 (red). Individual data points shown (females, black and males gray).

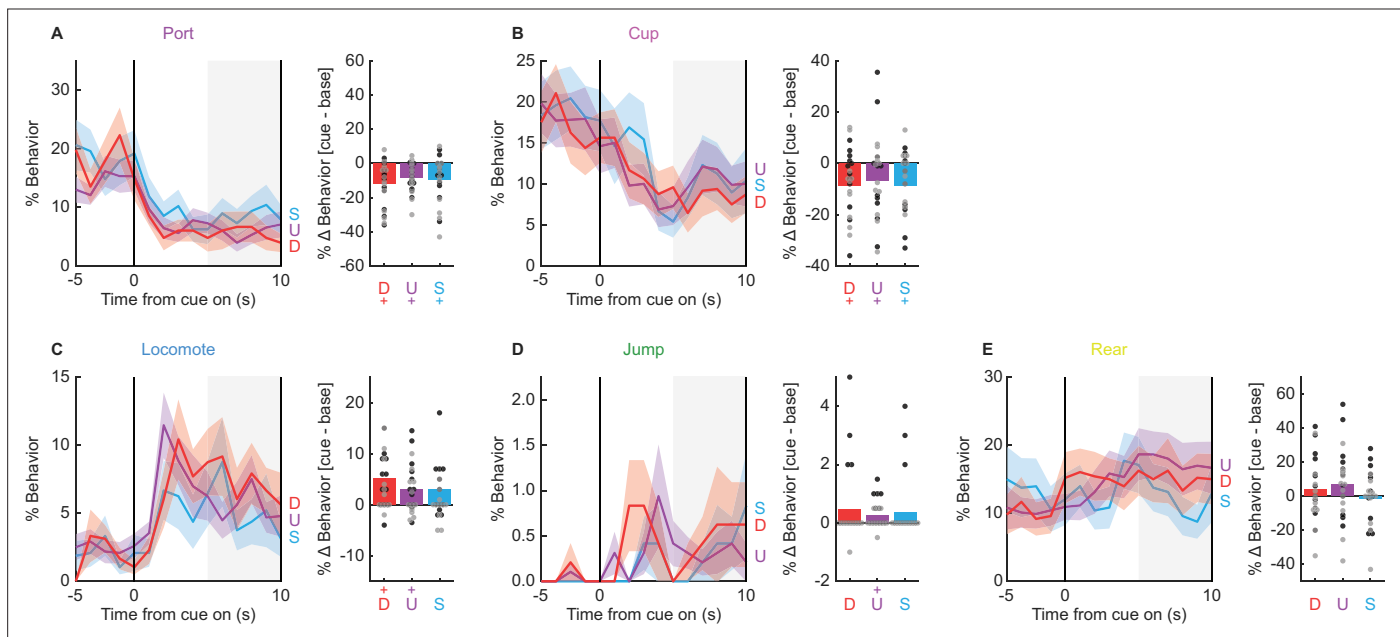


Figure 4—figure supplement 2. Session 16, danger-specific behaviors during session 2. Mean \pm standard error of the mean (SEM) percent behavior from 5 s prior through 10 s cue presentation is shown for (A) port, (B) cup, (C) locomote, (D) jump, and (E) rear, danger (red), uncertainty (purple), and safety (blue). Adjacent plots show mean % change in behavior from baseline to last 5 s of cue presentation for all rats. Individual data points shown (females, black and males, gray). +95% bootstrap confidence interval does not contain zero.

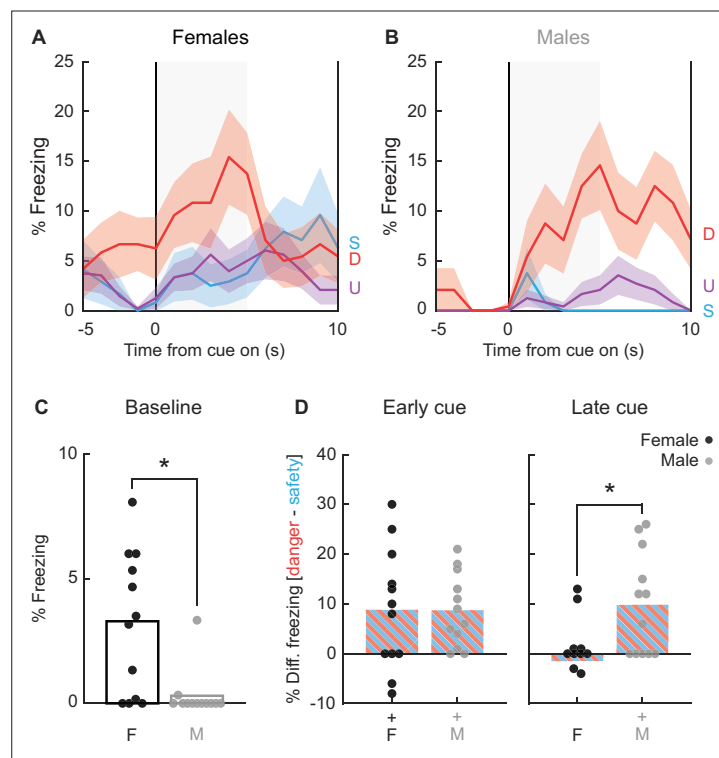


Figure 5. Special case of freezing. Line graphs show mean \pm standard error of the mean (SEM) percent freezing from 5 s prior through 10 s cue presentation for danger (red), uncertainty (purple), and safety (blue) for (A) females and (B) males. (C) Percent freezing during baseline (5 s prior to cue) is shown for females (black) and males (gray). (D) Mean differential freezing to danger and safety is shown for females (black, left) and males (gray, right) during early cue (first 5 s of cue, left) and late cue (last 5 s of cue, right). Mean \pm SEM percent freezing change from baseline (5 s prior to cue) compared to last 5 s of danger (red), uncertainty (purple), and safety (blue) for (E) females and (F) males.

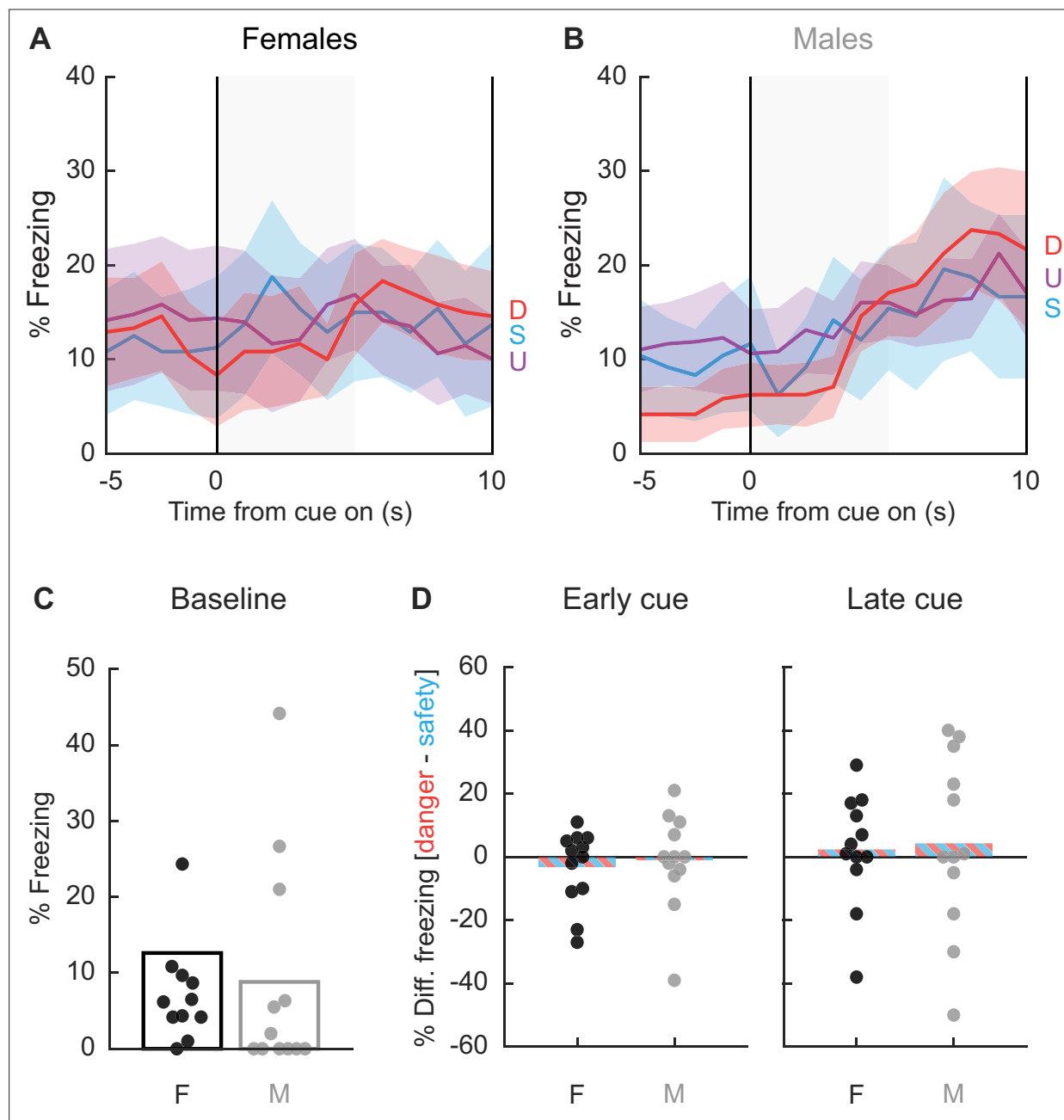


Figure 5—figure supplement 1. Session 2 freezing. Mean \pm standard error of the mean (SEM) percent freezing from 5 s prior through 10 s cue presentation is shown for danger (red), uncertainty (purple), and safety (blue) for (A) females, and (B) males. Analysis of variance (ANOVA) [factors: sex, cue, and interval] found no significant main effect or interaction for cue ($F_s < 1.4$, $p_s > 0.1$). Instead, ANOVA only revealed a significant main effect of interval and a significant sex \times interval interaction ($F_s > 2.5$, $p_s < 0.01$). Rats increased freezing to all cues over presentation and this increase was greatest in males. (C) Baseline freezing was equivalent in females and males and neither sex showed differential freezing to danger and safety during either early or late cue periods. Mean % differential freezing to danger and safety plotted (D). Individual data points shown (females, black and males, gray).

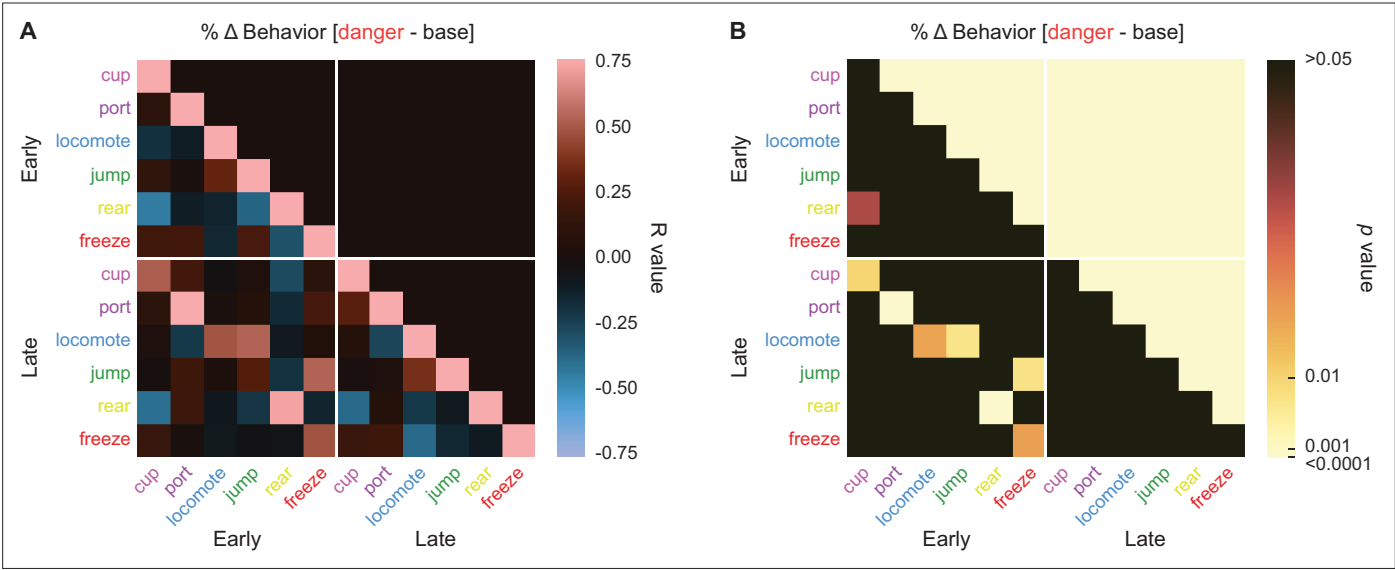


Figure 6. Behavior–behavior correlations. **(A)** A correlation matrix for the six cue-specific behaviors port (dark purple), cup (light purple), locomote (blue), jump (dark green), rear (yellow), and freeze (red) comparing mean percent behavior during early (first 5 s) and late (last 5 s) cue is shown. Lighter red values indicate positive *R* values, lighter blue values indicate negative *R* values. Black indicates *R* = 0. *p* values associated with each associated *R* value are shown in **(B)**. Black indicates *p* values greater than 0.05, while increasingly lighter values indicate lower *p* values.

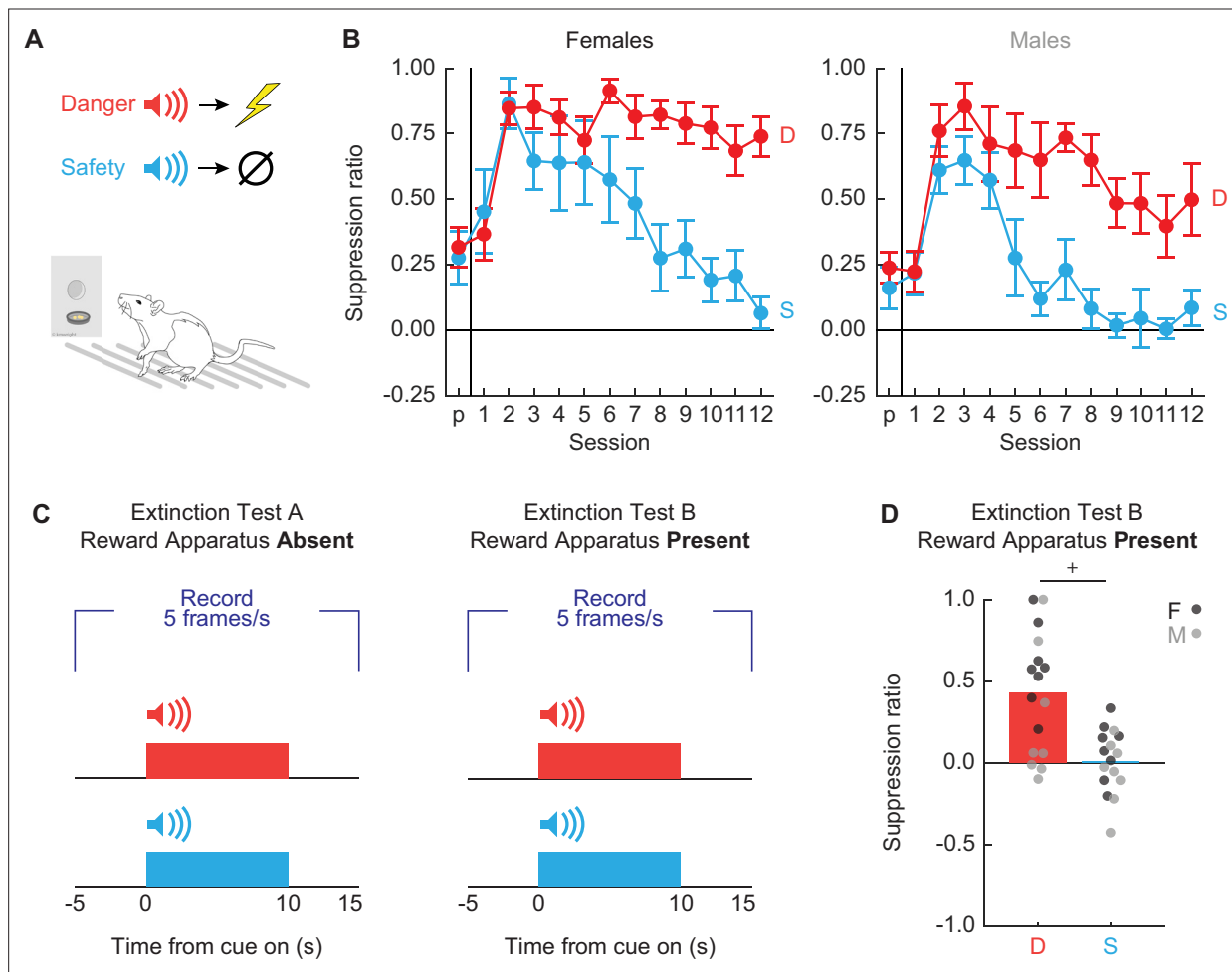


Figure 7. Experimental design and nose poke suppression. **(A)** Conditioned suppression procedure during which rats nose poke for food, while danger (red; $p = 1$) and safety (blue; $p = 0$) cues are played overhead and shocks delivered through floor. **(B)** Mean \pm standard error of the mean (SEM) suppression ratios for danger (red) and safety (blue) from pre-exposure through discrimination session 12 are shown for (left) females and (right) males. **(C)** Rats received one extinction test with reward apparatus absent (left), and another with reward apparatus present (right), counterbalanced. Five video frames were captured per second, starting 5 s prior to cue onset and continuing through 5 s after cue offset. **(D)** Mean + individual suppression ratios for each cue are shown for extinction with reward apparatus present. Individuals represented by black (female) and gray (male) dots. +95% bootstrap confidence interval does not contain zero.

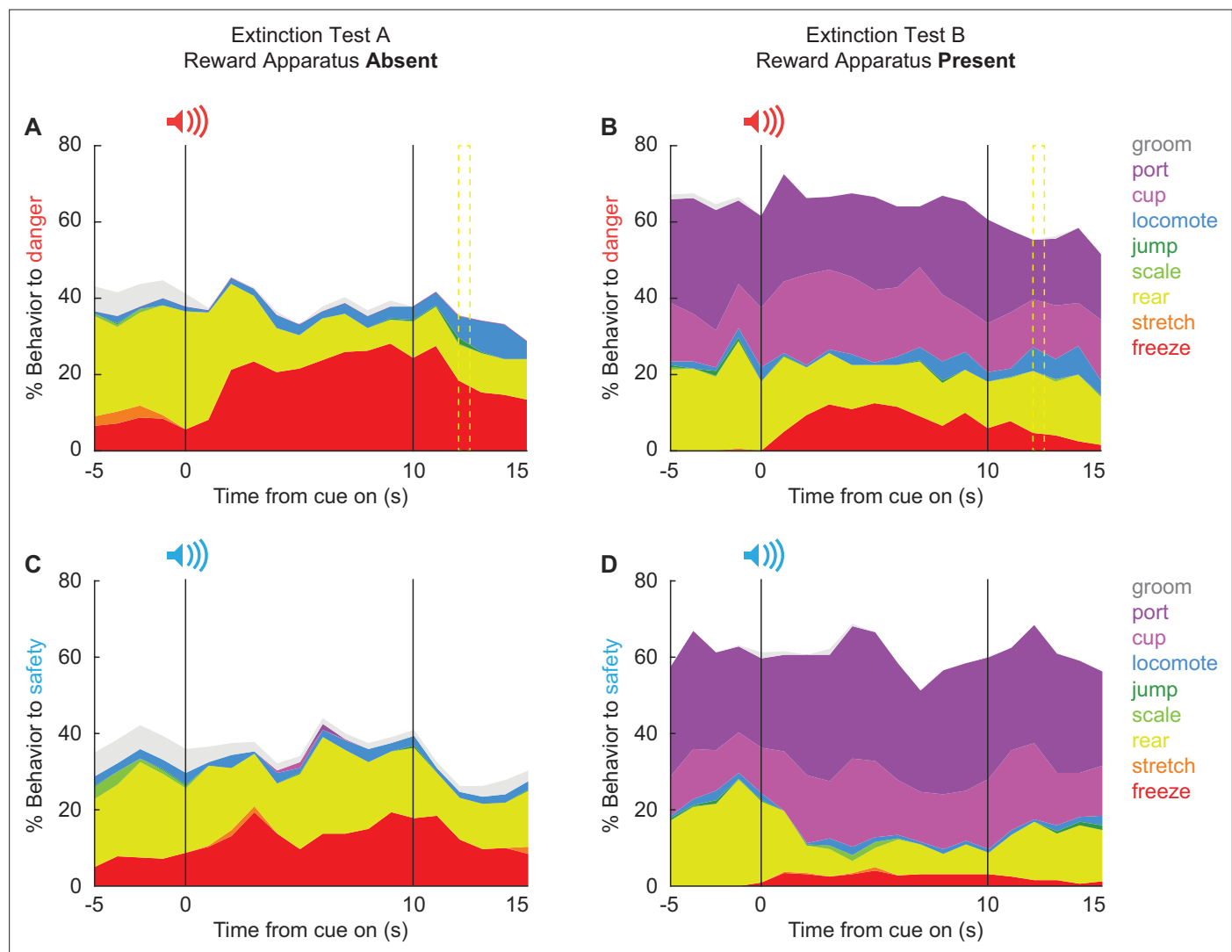


Figure 8. Temporal ethograms during extinction. Mean percent behavior from 5 s prior through 5 s following cue offset is shown for the danger cue during extinction with (A) reward apparatus absent and (B) reward apparatus present; and the safety cue during extinction with (C) reward apparatus absent and (D) reward apparatus present. Behaviors are groom (gray), port (dark purple), cup (light purple), locomote (blue), jump (dark green), scale (light green), rear (yellow), stretch (orange), and freeze (red). Note, port and cup are not shown for A and C because the food cup and nose port were absent.

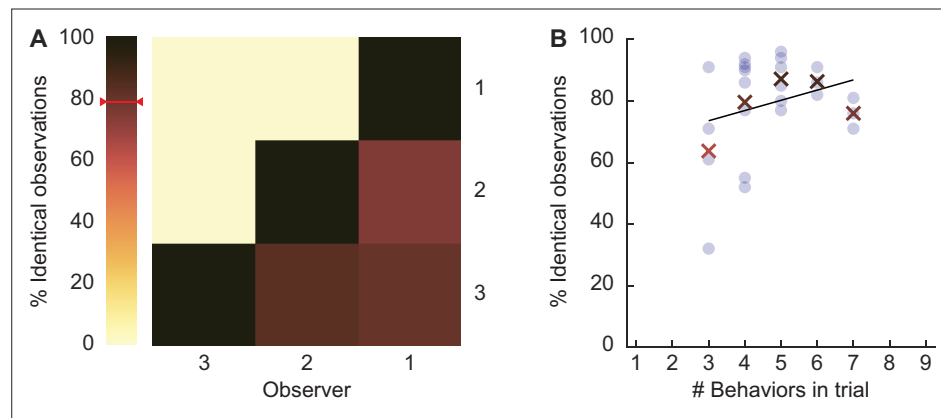


Figure 8—figure supplement 1. Inter-rater reliability. **(A)** Percentage of identical observations between observer–observer pairs. **(B)** Percentage of identical observations as a function of the number of behaviors present in a trial. Frames were systematically hand scored by three observers blind to rat identity, session number, and trial type (see Materials and methods for hand scoring approach and trial anonymization). A comparison dataset consisting of 8 trials (800 frames) was also scored by each observer. A correlation matrix compared % identical observations for the 800 comparison frames for each observer–observer pair. Mean % identical observation was 79.25%.

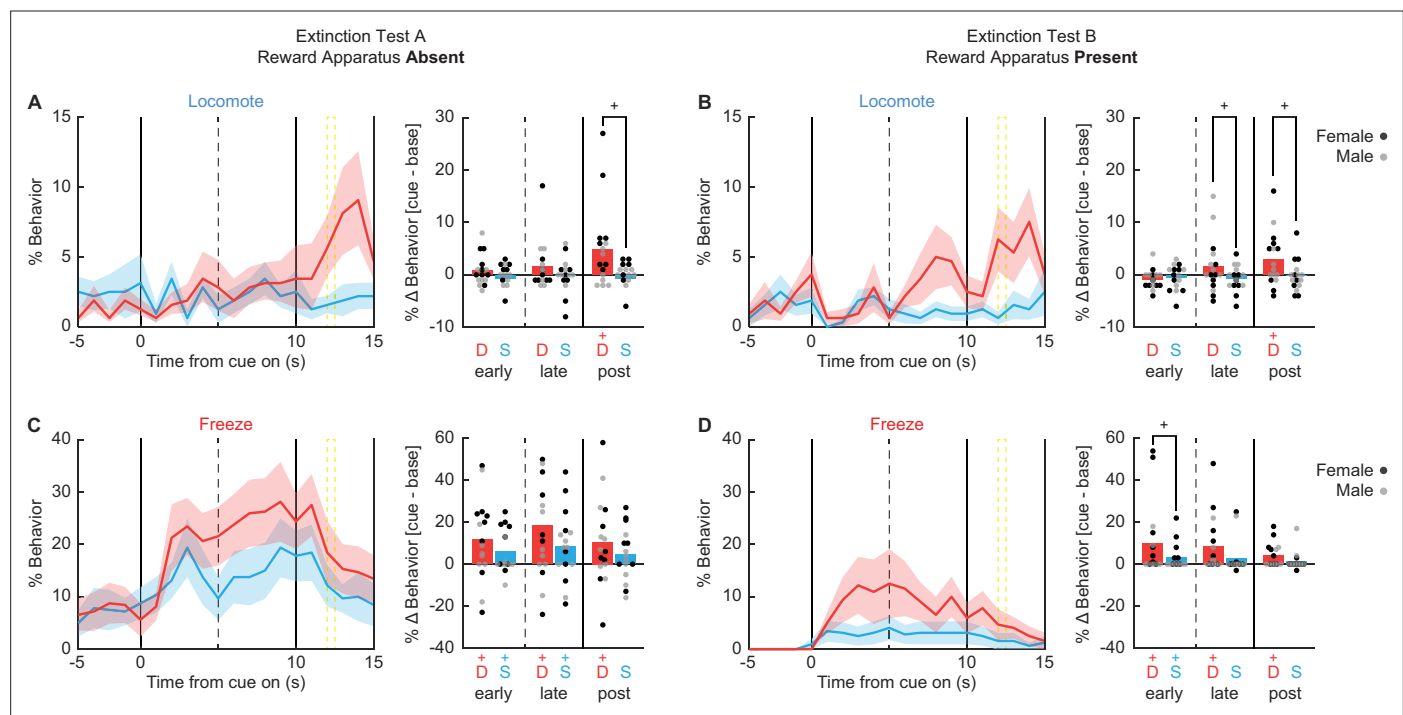


Figure 9. Danger elicits locomotion. Line graphs show mean \pm standard error of the mean (SEM) percent behavior from 5 s prior through 10 s cue presentation for danger (red) and safety (blue) for locomotion during the (A) reward apparatus absent extinction test and (B) reward apparatus present extinction test. Bar plots show mean change in behavior from baseline (5 s prior to cue) compared to early (first 5 s), late (last 5 s), and post (5 s after offset) cue periods. Individuals represented by black (female) and gray (male) dots. The same is shown for freezing (C, D). +95% bootstrap confidence interval for danger vs. safety (black), danger vs. baseline (red), or safety vs. baseline (blue) comparison does not contain zero (black).

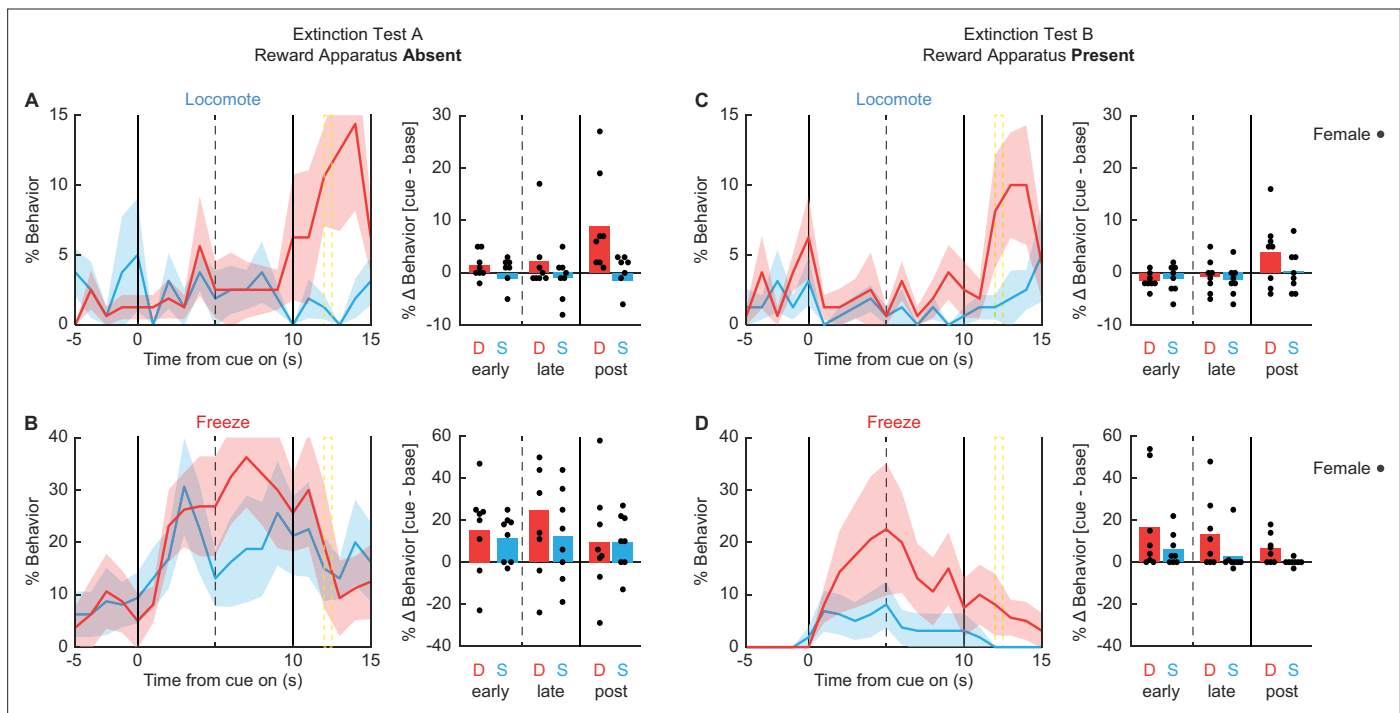


Figure 9—figure supplement 1. Female behavior during extinction testing. Line graphs show mean \pm standard error of the mean (SEM) percent behavior from 5 s prior through 10 s cue presentation for danger (red) and safety (blue) for locomotion during the (A) reward apparatus absent extinction test and (B) reward apparatus present extinction test. Bar plots show mean change in behavior from baseline (5 s prior to cue) compared to early (first 5 s), late (last 5 s), and post (5 s after offset) cue periods. Individual females represented by black dots. The same is shown for freezing (C, D).

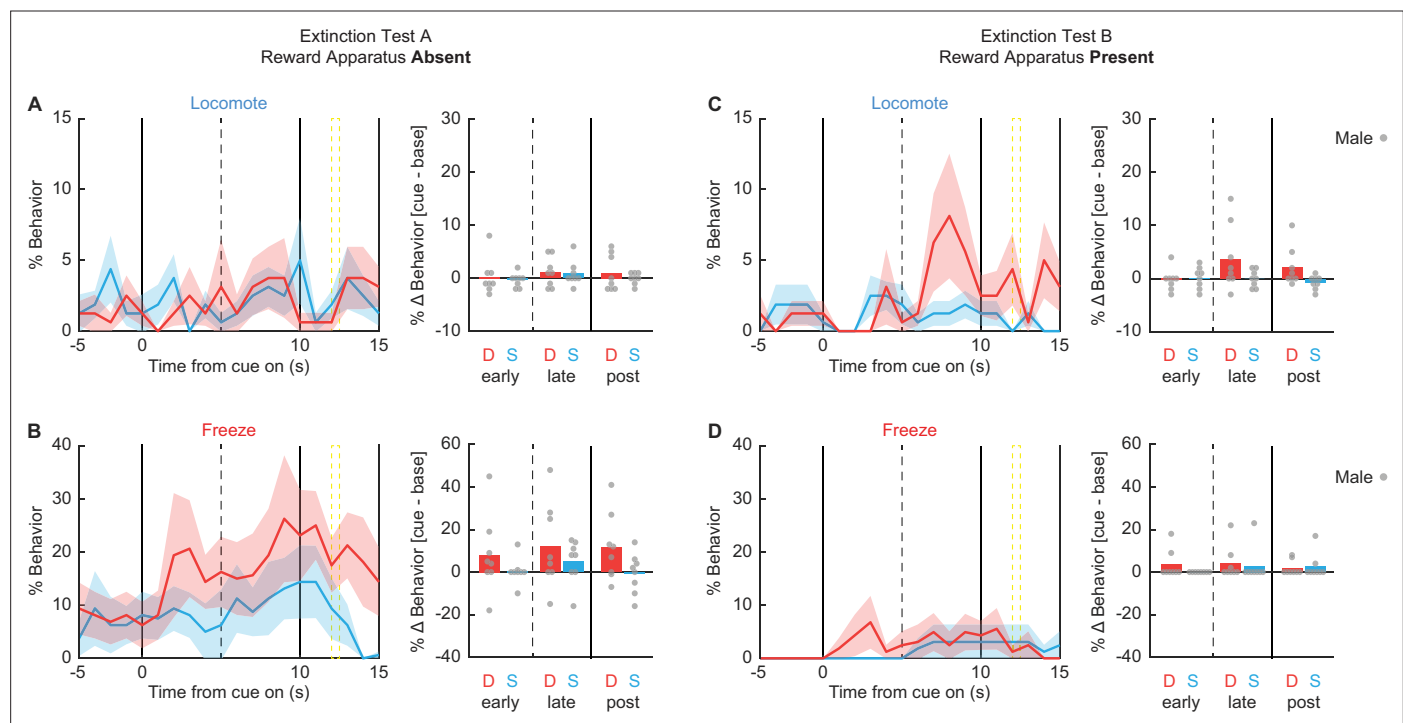


Figure 9—figure supplement 2. Male behavior during extinction testing. Line graphs show mean \pm standard error of the mean (SEM) percent behavior from 5 s prior through 10 s cue presentation for danger (red) and safety (blue) for locomotion during the (A) reward apparatus absent extinction test and (B) reward apparatus present extinction test. Bar plots show mean change in behavior from baseline (5 s prior to cue) compared to early (first 5 s), late (last 5 s), and post (5 s after offset) cue periods. Individual males represented by gray dots. The same is shown for freezing (C, D).

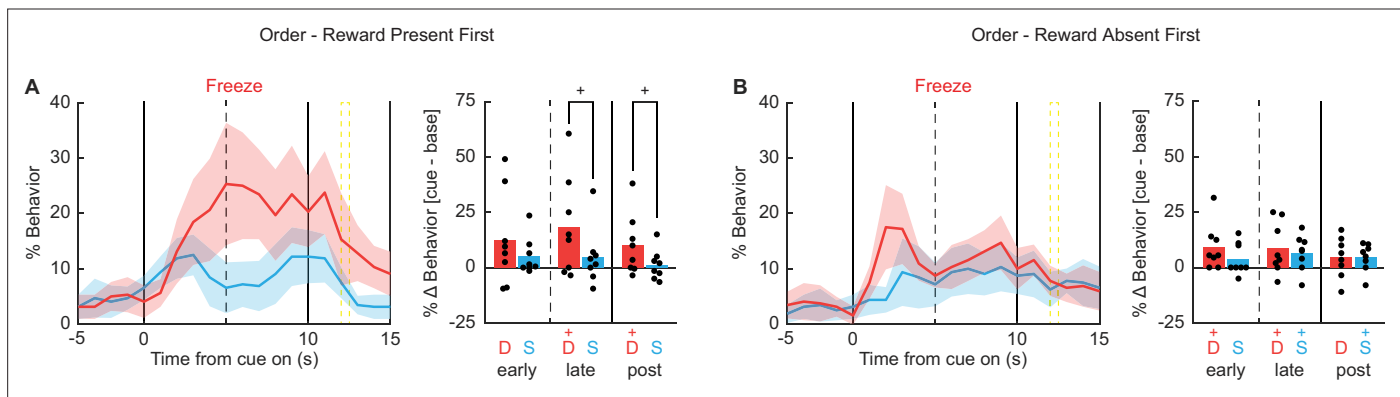


Figure 9—figure supplement 3. Freezing separated by test order. Line graphs show mean \pm standard error of the mean (SEM) percent freezing from 5 s prior through 10 s cue presentation for danger (red) and safety (blue) for rats receiving the reward apparatus present extinction test (A) first and (B) second. Mean responding is taken from both test types. Bar plots show mean change in behavior from baseline (5 s prior to cue) compared to early (first 5 s), late (last 5 s), and post (5 s after offset) cue periods. Individuals represented by black dots. +95% bootstrap confidence interval for danger vs. safety (black), danger vs. baseline (red), or safety vs. baseline (blue) comparison does not contain zero (black).

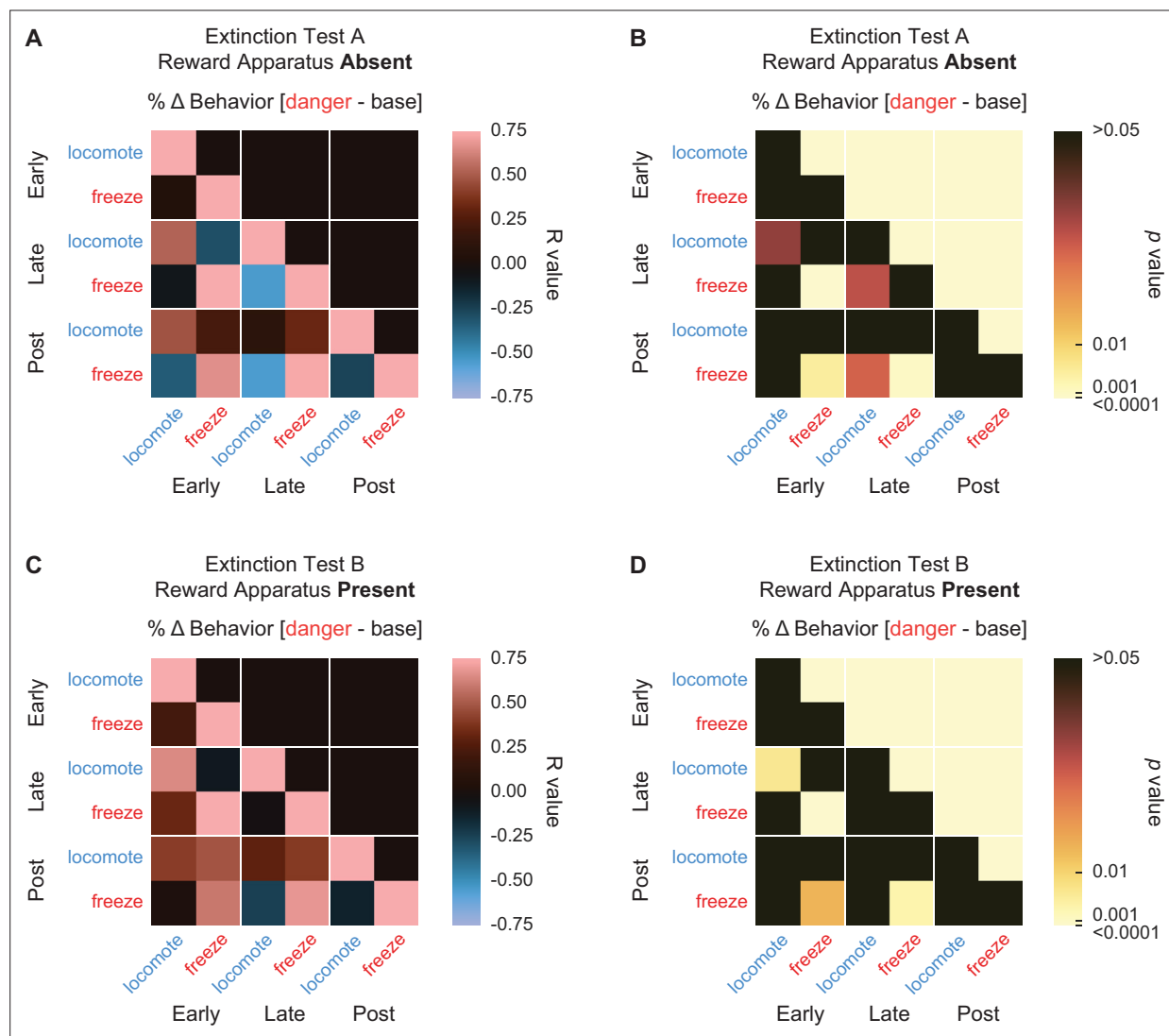


Figure 10. Behavior-behavior correlations during extinction. **(A)** A correlation matrix for locomote (blue) and freeze (red) comparing mean percent behavior during early (first 5 s), late (last 5 s), and post (5 s after) cue period is shown for the reward absent extinction test. Lighter red values indicate positive R values, lighter blue values indicate negative R values. Black indicates $R = 0$. p values associated with each associated R value are shown in **(B)**. Black indicates p values greater than 0.05, while increasingly lighter values indicate lower p values. Same shown for behavior correlations during reward present extinction test (**C**, **D**).