

Spontaneous behavioral responses during habituation epochs without a stimulus compared to stationary and moving stimulus presentation trials. (A) Mean number of freezes quantified for mice during last minute of habituation (No Stim), compared to each stimulus trial type by stationary stimulus size which were presented in a randomized order. Freezing was rarely observed in the absence of stimulus motion or in the absence of stimuli. No significant differences were found, one-way ANOVA, p > 0.05, N=10 mice. (B) Mean number of freezes quantified for mice during last minute of habituation (No Stim), compared to each stimulus trial type of a 2 x1 cm stimulus moving at three different speeds. Student's t-test, N=23. (C) Mean number of approaches to within 4 cm of the screened walls quantified for mice during last minute of habituation (No Stim), compared to each stimulus trial type of a 2 x1 cm stimulus moving at three different speeds. Student's t-test, N=23. (From Stim), compared to each stimulus trial type of a 2 x1 cm stimulus moving at three different speeds. Student's t-test, N=23. (From Stim), compared to each stimulus trial type of a 2 x1 cm stimulus moving at three different speeds. Student's t-test, N=23. (From Stim), compared to each stimulus trial type of a 2 x1 cm stimulus moving at three different speeds. Student's t-test, N=23. (From Stim), compared to each stimulus trial type of a 2 x1 cm stimulus moving at three different speeds. Student's t-test, N=23. (From Stim), compared to each stimulus trial type of a 2 x1 cm stimulus moving at three different speeds. Student's t-test, N=23. (From Stim), compared to each stimulus trial type of a 2 x1 cm stimulus moving at three different speeds. Student's t-test, N=23. Error bars are standard error of the mean (SEM).



**Freeze and approach frequencies by individual and by sex.** (A) The relative ratio of approaches to freezes for each individual also shows that most individuals freeze more and approach less when shown a faster speed of stimulus (increasing stimulus speeds top to bottom). (B) Approach (top) and freeze (bottom) frequencies are not significantly different between males and females. All scored behavioral responses to all three stimulus speeds of a 2x1 cm stimulus are shown for the females (lighter shading, N=15) and males (darker shading, N=8), Mann Whitney U, p > 0.0167, corrected for multiple comparisons. n.s. = not significant. If an individual did not exhibit a behavioral response of any kind to a specific stimulus, then that data point was excluded.



Summary of visual features correlated to specific moments of approach and freezing responses. (A) Trajectories of stimuli preceding and during approaches as represented by change in stimulus angle (head bearing relative to stimulus location) aligned to the onset of approach (Time = 0 s). Approach start marked by solid light grey line. Four random trajectories are bolded to show representative change in stimulus angle over time relative to the onset of an approach. Dashed lines represent 500 ms prior to approach start (-0.5 s) and 500 ms after approach start (0.5 s). (B) The predicted "mouse view" of the average stimulus over three distinct epochs for each approach: 1) average over the 500 ms prior to approach start (- 500 ms Start), 2) the stimulus at approach start (Start Approach) and 3) the average view of the stimulus over the 500 ms after approach starts (+500 ms Start). Stimulus is plotted as average angular size based on relative distance between stimulus and mouse versus visual stimulus angle, position in the probable visual field of the mouse. Central 40 deg of the visual field is highlighted with light grey shading, green is naïve and magenta prey capture experienced mice. Histograms representing the probability of a stimulus occupying a particular visual stimulus angle during each epoch are also plotted. (C) Graphical summary of representative stimulus features preceding approaches (-500 ms through start) versus during approach (from approach start through the duration of the approach). Stimuli are depicted within the rectangle as though from the view of the mouse shown below: arrows depict stimuli moving towards the central visual field (0°) of the mouse from the periphery (towards 90°), and surrounding brackets indicate radial expansion the stimulus with the central visual field. Note, preferences are represented, but they do not exclusively predict the behavior shown. (D) Trajectories of stimuli preceding and during freezes as shown in A. Stimulus trajectories are aligned to onset of freeze starts (Time =

0 s). (E) The predicted "mouse view" of the average stimulus over three distinct epochs for each freeze as in B. (F) Graphical summary of representative stimulus features preceding freezes (-500 ms through start) versus during freeze (from freeze start through the duration of the freeze). Dark grey data are from naïve mice and blue from prey capture experienced mice, arrows indicate that stimuli motion mostly moves towards the periphery (>90°) during a freeze. \*Either rightward or leftward stimulus motion is observed by the mouse with similar frequencies during freezing, though leftward motion is shown in the graphical summary).



Mice with prey capture experience strongly prefer to approach moving stimuli with speeds less than 50 cm/s. (A) Top, an ethogram representing when approaches and freezes occurred from the onset of presentation of three different speeds of the stimulus for each mouse. Magenta = approaches by prey capture-experienced mice, grey = freezing displayed by prey capture-experienced mice. N = 23 mice performing one trial at each of three stimulus speeds, dashed line separates speed trial types. Bottom, histogram showing the proportion of time spent engaged in each type of response during each 5 s segment of the 60 s trial collapsed across all speeds. The distribution of approaches is bimodal and 26 s separates the modes (vertical grey dashed line). (B) Mean time to the first behavioral event of each type for mice that displayed at least one type of orienting response (two-way ANOVA, (F(1) = 9.1957, p)< 0.06, Tukey's post hoc, # = 0.0745, Ns = 13, 19, 6 mice and 15, 20, 20 mice for each speed, approaches versus freezes, respectively. (C) Percentage of trials at each speed with freezing (grey) or approach (magenta) showing significant differences in the observation of each type of behavior and that freezing probability steadily increased with objective stimulus speed (Fisher's Exact test, \* = p < 0.05 and \*\* = p < 0.001 and adjusted  $r^2 = 0.89$ ). Error bars are standard deviation. (D) Number of approaches per mouse (one-way ANOVA, (F(2) = 9.68, p < 0.001, Tukey's post hoc, \* = p < 0.05 and \*\*\* = p < 0.001). (E) Number of freezes per mouse. The effect of stimulus speed on the number of freezes observed during a trial between speeds approached significance (# = p=0.072). (F) Approach-to-freeze index calculated as shown in Figure 2 (one-way ANOVA, (F(2) = 13.11, \*\*\*p < 0.0001, Tukey's post hoc, \*\*\* = p < 0.0001). Error bars are +/- SEM.



**Prey capture-experienced mice prefer to approach objects with distinct visual features from those that drive freezing.** (A) Distribution of mouse ranges (cm) and stimulus angles (deg) relative to the head bearing where freezes occur (grey) or approaches start (magenta). The range and stimulus angle distributions for approaches versus freezes are shifted relative to each other, showing opposite skews towards far ranges for freezes and near ranges for approaches (KS test, p < 0.01). We observe a bimodal distribution of stimulus angles towards the periphery for freezes, and normal distribution well-centered near 0 deg for approaches. All individual behavioral events for all 23 mice are plotted (N = 74 and 224, approaches versus freezes, respectively). (B) Mean trial-averaged range at all three speeds where approaches started (magenta circles), or freezing occurred (grey circles) (two-way ANOVA, (F(1) = 12.49, p < 0.001, Tukey's post hoc, \* = p < 0.05, Ns = 13, 19, 6 mice and 15, 20, 20 mice for each speed of stimulus, approach versus freezing data, respectively). (C) Mean trial-averaged, absolute head angles of the mouse relative to the stimulus where approach started or freezing occurred showing that approaches were initiated at more

direct stimulus angles than freezes, and this separation increased as stimulus speed increased (two-way ANOVA, (F(1) = 44.39, p < 0.0001, Tukey's post hoc, \* = p < 0.05) (D) Angular size versus angular speed of stimuli at approach starts (green) or freeze (grey) reveals a difference in stimulus features that precede approaches versus freezes.



**Mice do not perceive moving stimuli as threatening.** Mean trial averaged percent time spent within 10 cm from the periphery of the arena (thigmotaxis) across mice that displayed at least one approach start.