

# Vision guides selection of freeze or flight defence strategies in mice

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## Keywords

mouse; innate behaviour; predator and prey; visual pathways

## Summary

In prey species such as mice, avoidance of predators is key to survival and drives instinctual behaviours like freeze or flight [1, 2]. Sensory signals guide the selection of appropriate behavior [3], and for aerial predators only vision provides useful information. Surprisingly, there is no evidence that vision can guide the selection of escape strategies. Fleeing behavior can be readily triggered by a rapidly looming overhead stimulus [4]. Freezing behavior, however, has previously been induced by real predators or their odors [5]. Here we discover that a small moving disk, simulating the sweep of a predator cruising overhead, is sufficient to induce freezing response in mice. Looming and sweeping therefore provide visual triggers for opposing flight and freeze behaviours, and provide evidence that mice innately make behavioural choices based on vision alone.

## Results

For a foraging mouse, a rapidly expanding overhead stimulus suggests the approach of a predator that has detected it. To avoid capture, rodents typically flee to an available refuge [4, 6]. But what if the potential predator is instead cruising overhead, as if unaware of the mouse? Flight or sudden movement would raise the risk of being detected, whereas freezing may promote survival. Here, we characterised the behaviour of mice during such distal threats.

We first confirmed that mice flee an imminent, looming threat (Figure 1A). To do this we placed a mouse in a rectangular arena with an opaque refuge in one corner (Figure 1C). A computer monitor placed on top of the arena displayed a blank grey screen. After habituating the mouse to the arena for 15 minutes, we triggered a visual stimulus when the mouse passed near the centre of the arena. The 'loom' stimulus was a black disk rapidly widening to 50 degrees of visual angle in 250 ms (Figure 1A). As expected, presentation of this stimulus reliably caused mice to flee to the refuge (Figures 1D and 1G; Movie S1). To quantify this behaviour we defined flight as epochs where the mouse returned to the refuge

at speeds exceeding 40 cm/s (Figure S1A). Flight was observed in 87.8% of loom presentations (79/90 trials in 28 mice; Figure 1G).

We found an opposing response to a distal threat. The 'sweep' stimulus was a small black disk that appeared at a corner of the monitor and moved smoothly across it for 4 seconds (Figure 1B). The stimulus emulates a 2m wide predator, flying 25m above the animal at 34 km/hr – a visual speed of 21 °/s to a mouse underneath it. The movement speed of the mice substantially decreased during the sweep stimulus (Figures 1E and 1H; Movie S1), and included epochs of complete immobility. These data were obtained from animals that had only ever been exposed to the sweep stimulus. As a quantitative measure of freezing we identified epochs in which mouse speed was less than 2 cm/s for at least 0.5 s. Freezing was observed in 83.6% of the sweep presentations (56/67 trials in 38 mice, Figure 1H). By contrast, flight occurred in 22.4% of trials (15/67 trials) - in 9 of these, the animal froze before fleeing. Freezing behaviour was similar for white and black sweep stimuli (Figure S2).

Mice sometimes pause while foraging, or return to the refuge, even in the absence of a real threat. To estimate the frequency of these stimulus-independent behaviours we analysed the last 5 minutes of the habituation period (before any visual stimulus), analysing only those epochs where the animal approached the centre of the arena, and applying the same criteria used above (Figures 1F and 1I). We found that the 'chance' probability of freeze was 0.13, and of flight was 0.01. The stimulus-induced effects we observed above were much greater than this ( $p < 10^{-10}$  for both freeze and flight, Binomial test).

The speed of a distal threat might influence behavioural response and we therefore asked if mice are sensitive to the speed of the sweep. In a new cohort of 10 mice we presented sweeps of varying speed (5, 21, 42 or 84 °/s). The standard sweep speed (21 °/s, Figure 2B) produced responses similar to that in the cohorts described above. Slower speeds (5 °/sec, Figure 2A) led to robust freezing behaviour (Movie S2), occasionally with long-latency flight. Faster sweep stimuli (42 °/s; Figure 2C) led to freezing behaviour, with increased probability of flight. During presentation of the fastest sweep (84 °/s; Figure 2D), however, we observed a strikingly different pattern of responses: mice showed rapid flight behaviour (latency  $705 \pm 163$  ms, mean  $\pm$  s.e.m.; median = 549 ms;  $n = 9$  flights in 10 trials), reaching movement speeds similar to those evoked by loom stimuli (Figure 2G). The latency to flight is longer than those evoked by loom stimuli ( $218 \pm 16$  ms, median = 199 ms;  $n = 41/47$ ), and pattern of movements around flight onset was quite different: fast sweeps were associated with a brief reduction in movement speed before flight commenced, but looms were not (Figure 2G; Movie S2).

Does freezing behaviour impede subsequent flight, and thereby account for the different flight latencies for loom and fast-sweep stimuli? To assess this we presented the sweep stimulus and then the loom stimulus in succession (Figure 3A), using new cohorts of mice. Using the trials where mice remained in the arena until onset of the loom stimulus (65/82 trials), we were able to estimate the effect of a preceding sweep stimulus on probability and latency to flight. The probability of flight to the looming stimulus (53/65 trials, 81.5%; Figure 3B,C) was similar to that in absence of a preceding sweep stimulus. Latency to flight after onset of loom stimulus was  $250 \pm 33$  (median = 159 ms;  $n = 53$ ), not significantly

different to that observed in absence of a preceding sweep stimulus. This implies that engaging one motor action (freezing) does not interfere with activation of another (flight).

## Discussion

Our results reveal that mice naturally select between possible defensive behaviours based on vision alone. To our knowledge this is the first evidence that variation in a single sensory modality is sufficient to select between opposing freeze and flight behaviours, and a clear demonstration of the utility of vision for mice. Previous attempts to influence the choice of freeze and flight behaviours [5, 7] have had to rely on presenting real predators [5], which inherently produce multisensory cues, or changing the availability of refuge [8].

The different defensive behaviours might be mediated by distinct visual pathways. Specialised circuits for loom-induced flight emerge early in visual processing in many species [9-13], potentially as early as the retina [4]. It is generally thought that the mammalian superior colliculus is important in behavioural response to loom stimuli [3, 14]. The sweep-induced behaviours that we observe might also be mediated by specialised subcortical pathways. For example, recent work shows a class of neurons in the mouse superior colliculus ('widefield cells'), which respond to small moving stimuli over a large region of the visual field [15]. Cortical contributions to defensive behaviours are also likely, as visual cortical projections to superior colliculus in mouse both modulate visual responsiveness [16] and help drive temporary arrest behaviours [17].

Flight behaviour can be rapid and reproducible following loom stimuli. However, the variable latency to flight during presentation of sweep stimuli (eg. Figures 2A-C), the direct path back to refuge, and the fact that flights are less likely when refuge is unavailable [8], suggest that flight behaviour is not a simple reflex. Further, flight behaviours can be initiated even whilst freezing (eg. Figure 2G). This suggests that during freezing behaviour, mice are engaged in sustained assessment of their defence strategies, allowing deliberation and selection of an optimal strategy. Defining an optimal defence strategy requires considering factors such as the availability and potential path to a refuge, the trajectory of the predator, and its velocity [9, 18-21]. Indeed, we observed that mice were more likely to engage flight during faster sweep stimuli.

We demonstrate a simple way to drive opposing avoidance behaviours through easily controlled visual stimuli. Combined with the availability of genetic tools in mice, this new framework may help better understand how this selection is made, as well as the visual processing [22] and sensorimotor integration that supports these decisions.

## Experimental Procedures

All procedures were conducted in accordance with the UK Animals Scientific Procedures Act (1986). Experiments were performed at University College London under personal and project licenses released by the Home Office following appropriate ethics review.

### Environment & Visual Stimulation

The behavioural arena was a 48 cm wide x 35 cm deep x 30 cm high box. An opaque triangular refuge 20 cm wide x 12 cm high was positioned in one corner. Visual stimuli were generated using the freely available software Expo (P. Lennie) and presented on a calibrated LCD monitor displaying a grey screen (48 cm x 27 cm, mean luminance 30-40 candela/m<sup>2</sup>, refresh rate 60 Hz, Asus) that filled most of the open top of the arena. Mouse movements were video-recorded with a camera (DMK 22BUC03, Imaging Source, sampling rate 60 Hz; except in cohort 'a', described below, where it was a Creative HD USB, sampling rate 30 Hz; this cohort was excluded from latency calculations), fitted with a wide-angle lens and positioned over the arena. Frames were acquired continuously in Matlab (Mathworks, Natick, MA) and temporally aligned to visual stimulus by simultaneously acquiring (via a Labjack U6, sample rate 1 kHz) the response of a photodiode to synchronous visual stimuli presented in a corner of the monitor that was obscured from the animal.

The 'loom' stimulus was a 1 cm (thus a visual angle of diameter 2° when directly over the animal) black disk rapidly widening to 25.5 cm (50°) in 250 ms, and remaining on the screen at this size for an additional 500 ms. The standard 'sweep' stimulus was a 2.5 cm (5°) black disk that appeared at a corner of the monitor and then translated smoothly to the diagonally opposite corner over 4 seconds (21 °/s). In some experiments the same black disc instead moved across the monitor in 16 s (5 °/s), 2 s (42 °/s), or 1 s (84 °/s), or was a white disc of the same size and moving at the standard speed (21 °/s). The 'sweep + loom' stimulus was also a 2.5 cm black disk, that appeared on the short edge of the monitor and translated along the midline for 2.6s, by which time it had traversed 32 cm from the starting edge of the monitor. The disk then expanded (loom) to 25.5 cm either from the same position, or on the other side of the monitor (16 cm from the starting edge).

### Testing

Prior to the first trial, animals were allowed to habituate to the arena for 15 minutes; in subsequent trials, the habituation period was 5 minutes. After habituation, a visual stimulus was triggered when the animal's location was approximately under the centre of the monitor. One trial was conducted each day, except in one cohort of animals ('a', defined below) where the loom stimulus followed the sweep stimulus by at least 1 minute.

### Cohorts

A total of 65 adult mice were housed under 12:12 light/dark cycle and tested during the dark period. Cohort 'a' (Figure S1A and S1B) was 8 male adult wild-type mice (C57BL/6, aged 13 - 18 weeks), and was tested once for the sweep stimulus, and then 6 times for the loom. Cohort 'b' (Figure S1A and S1B) was 10 male adult wild-type mice (C57BL/6, aged 11 -12 weeks), that were tested 4 times for the sweep stimulus (the first encounter is indicated by 'b1', subsequent encounters are indicated by 'b2') and then 3 times for the loom stimulus. Cohort 'c' (Figure S1C) was 18 adult wild-type mice (C57BL/6, 4 female, aged 8 -10 weeks), tested 4 (8 animals) or 5 (10 animals) times for the sweep + loom stimulus. Ten of the animals were also tested 2 times for the loom stimulus. In the sweep + loom trials, the looming disk expanded from either the final position of the sweep (cohort 'c2') or from an alternative location of the sweep trajectory (cohort 'c1'). Cohort 'd' (Figure S1B) was 19 mice housed and tested in a different facility, and included animals of different ages and genetic profile. 11 animals were adult male Gad2Cre on C57BL/6 background (aged 6-42 weeks), 6

were adult wild-type mice (C57BL/6, aged 8 weeks with an exception of 43 weeks), 2 were of other genetic profiles on C57BL/6 background (aged 7-9 weeks). Subdividing this cohort in animals aged 13 weeks or less (n=14), more than 28 weeks (n = 5), or Gad2Cre genetic profile (n = 11) showed no differences in freezing probability after the sweep stimulus (78.6%, 78.6% and 81.8% respectively). Cohort 'e' was 10 male adult wild-type mice (C57BL/6, aged 7-8 weeks), that were tested with black sweep stimuli of different speeds (5, 21, 42 and 84 °/s), and a white sweep stimulus of speed 21 °/s, in 6 sessions. The order of stimuli was randomised for each mouse.

### Analysis

The position of the animal during the experiment was extracted from video recordings using custom software in the Matlab environment. Manual thresholds were set to identify pixels over the mouse in each video, and the centre-of-mass of these pixels was used to define mouse position on each frame. The wide-angle and oblique orientation of the camera lens introduces barrel and projective distortions in the image. We estimated this distortion by calculating the requisite polynomial transformation matrix from daily calibration images using the function *cp2tform* in Matlab. The inverse of this matrix was used to transform positional estimates from image space to arena space, using the function *tforminv*. Transformed positions were accurate to within 1.5 mm. Inspection of responses to loom stimulus suggested that flights could be defined as periods of time during which the mouse speed was higher than 40 cm/s and the animal returned to the refuge within 1 second following the onset of this movement. Freezes were defined as periods of time during which the speed decreased to less than 2 cm/s for at least 0.5 seconds. Average speed across trials was calculated as the geometric mean and the s.e.m. of the geometric mean. For baseline measurements, we analysed activity prior to presentation of visual stimulus. We analysed 4s video sequences that were triggered on the animal moving away from the walls towards the centre of the arena. Latency of flights was defined as the time from the onset of a stimulus to the time at which movement speed had increased by 20cm/s above that at stimulus onset (response on 1 loom trial did not reach this criterion). Latency was not clearly correlated with movement speed at time of loom onset ( $r = -0.02$ ,  $p = 0.82$ ,  $n = 94$ ). For display purposes, we filtered the speed traces with a moving average filter of width 83 ms.

### **Author Contributions**

The experiments were conceived by A.B.S. and S.G.S., and designed by G.D.F., A.B.S. and S.G.S. The behavioural monitoring software was written by G.D.F. and S.G.S. Experiments were conducted by G.D.F. and T.V., and data was analysed by all authors. G.D.F., A.B.S. and S.G.S. wrote the paper.

### **Acknowledgements**

We thank E. Bracey and T. Margrie for help in experiments, H. Rashid for help with pilot experiments, K. Jeffery for useful discussions and E. Arabzadeh, D. Bendor, J. Fournier and H. Spiers for comments on previous versions of the manuscript. SGS received support from the National Centre for the 3Rs (NC/L000385/1) and the Marie-Curie scheme of the European Commission (PVPITM).

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## Figure legends

### **Figure 1: Visual stimulus-dependence of freeze and flight behaviours in mouse. A-B.**

Schematics of visual stimuli. The loom stimulus expanded from 1 to 25.5 cm ( $2-50^\circ$ ) in 250ms, and persisted for 500ms. The sweep stimulus was a 2.5 cm ( $5^\circ$ ) diameter black disc translating across the monitor at an angular speed of ca.  $21^\circ/\text{s}$  for 4s. **C.** Schematic of the experimental arena. A computer monitor was placed on top of the arena. An opaque triangular refuge was provided in a corner. A camera video-recorded the movements of the mouse. **D-F.** Upper panels: images of the natural logarithm of movement speed in each trial (one trial per row). Red indicates low speed; green indicates high speed; black indicates speeds close to the mean across animals, and white indicates times when the animal was in the refuge. Lower panels: mean ( $\pm 1$  s.e.m.) movement speed of mice across trials. Traces clipped after flight home. Horizontal dashed lines indicate mean  $\pm 1$  s.e.m. of movement speed in absence of visual stimuli, as shown in panel F ('BASELINE'). **G-I.** Cumulative probability of having observed a flight (green) or freeze (red) response over time. See also Figure S1 and Movie S1.

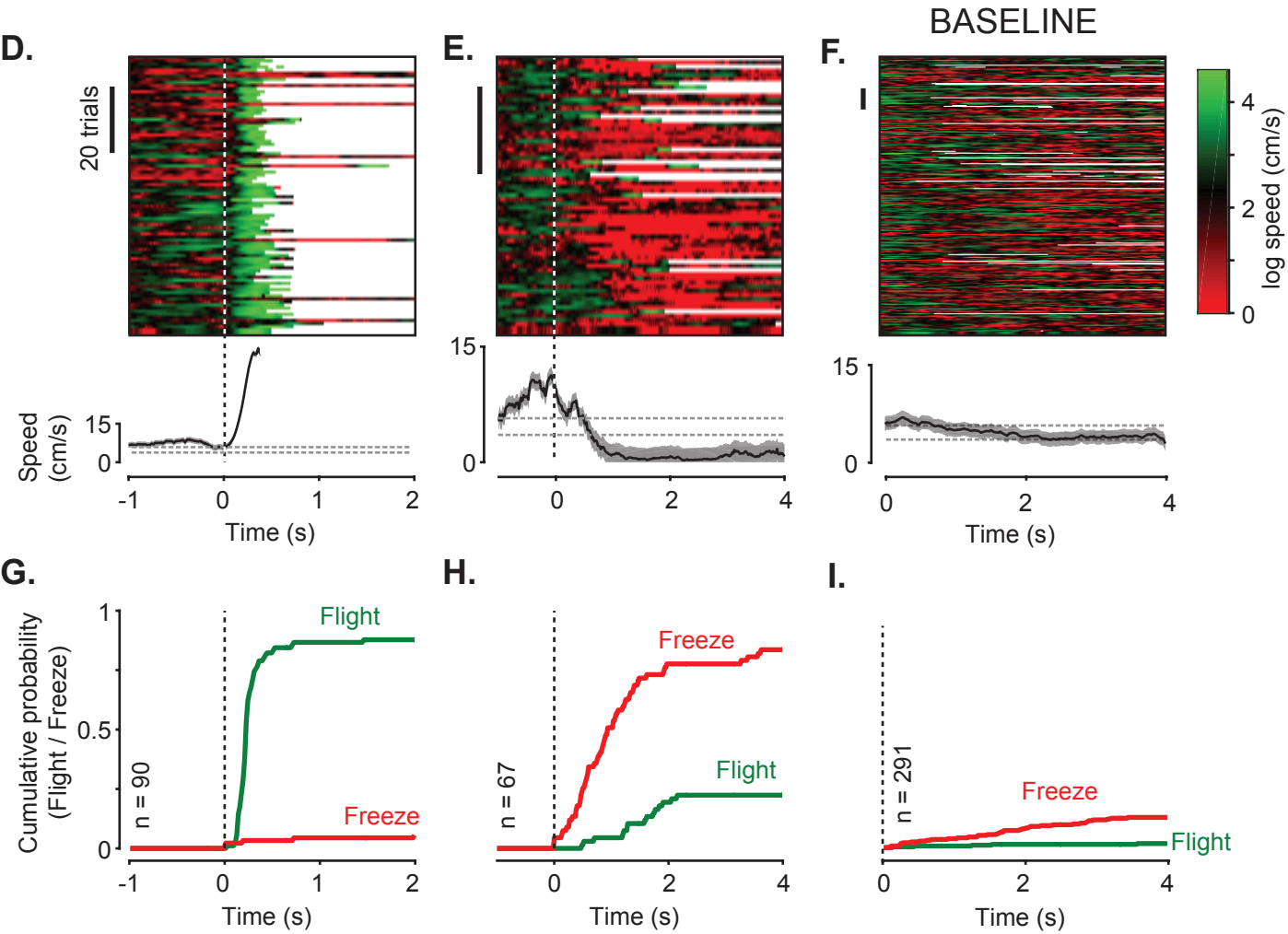
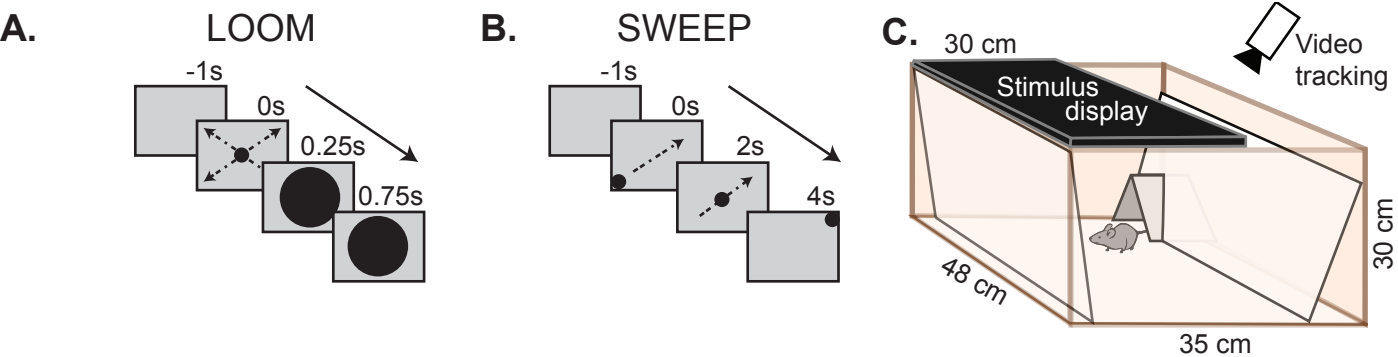
### **Figure 2. Dependence of freeze and flight behaviours on stimulus speed. A-D.**

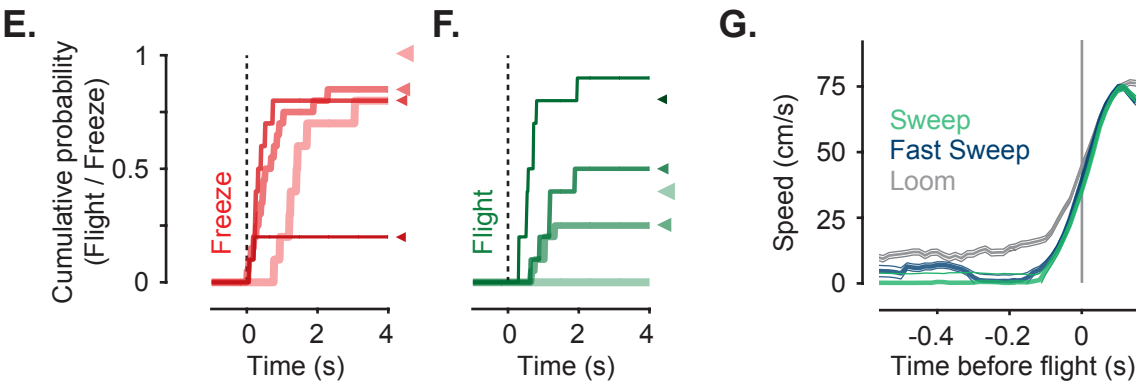
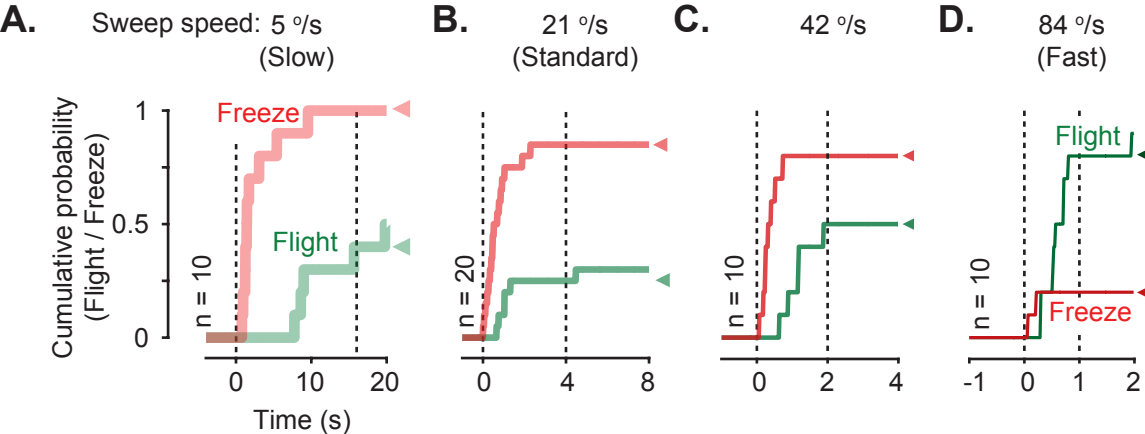
Cumulative probability of having observed a flight (green) or freeze (red) response during presentation of black sweep stimuli of varying speed. Vertical dashed lines indicate the start and end of the stimulus from the monitor. Triangles indicate probability at stimulus end. Duration of stimulus presentation depends on stimulus speed. **E.** Cumulative probability of observing a freeze response at each speed (5, 21, 42 and  $84^\circ/\text{s}$ ), over the first 4s of stimulus presentation. Thickness of the line indicates stimulus speed, as in A-D, with thickest lines showing slowest speed. Triangles replotted from A-D show probability at stimulus end. Vertical dashed line indicates start of stimulus. **F.** Same as E, but for flight response. **G.** Mean ( $\pm 1$  s.e.m.) of movement speed around the time of flight responses during presentation of standard sweep ( $21^\circ/\text{s}$ ,  $n = 6$  flights from 20 trials), fast sweep ( $84^\circ/\text{s}$ ,  $n = 9/10$ ), or loom stimulus ( $n = 42/47$ ). Speed traces were aligned to the time at which movement speed exceeded 20 cm/s of the speed at stimulus start (vertical line). See also Movie S2.

### **Figure 3. Behavioural responses to combinations of sweep and loom stimuli. A.**

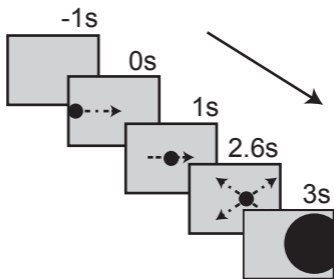
Schematic of visual stimulus. The standard sweep stimulus ( $21^\circ/\text{s}$ ) was presented for 2.6s, and was immediately followed by a loom stimulus. **B.** Upper panel: images of the natural logarithm of movement speed in each trial (one trial per row). Red indicates low speed; green indicates high speed; black indicates speeds close to the mean across animals, and white indicates times when the animal was in the refuge. Lower panel: mean ( $\pm 1$  s.e.m.) movement speed of mice across trials. Horizontal dashed lines indicate mean  $\pm 1$  s.e.m. of movement speed in absence of visual stimuli, as in Figure 1. **C.** Cumulative probability of having observed a flight (green) or freeze (red) response over time. See also Figure S1 and Movie S1.



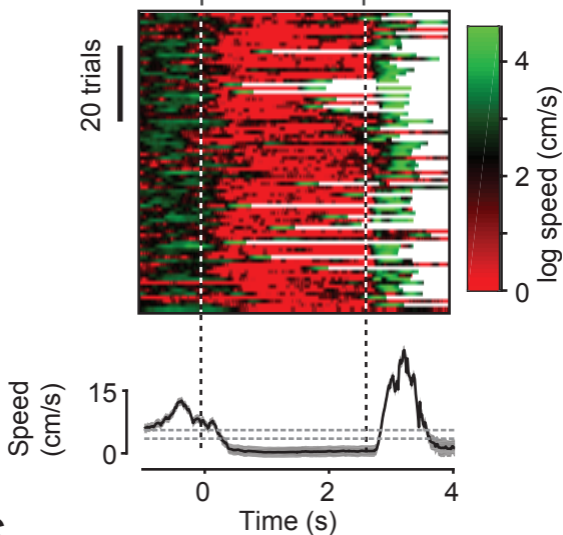




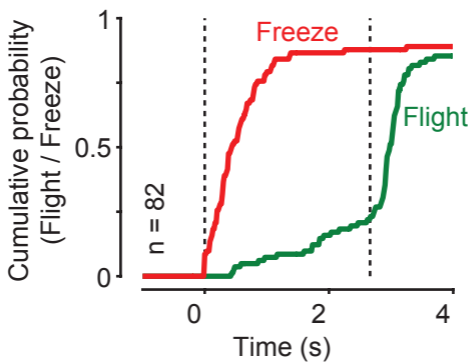
# A. SWEEP + LOOM



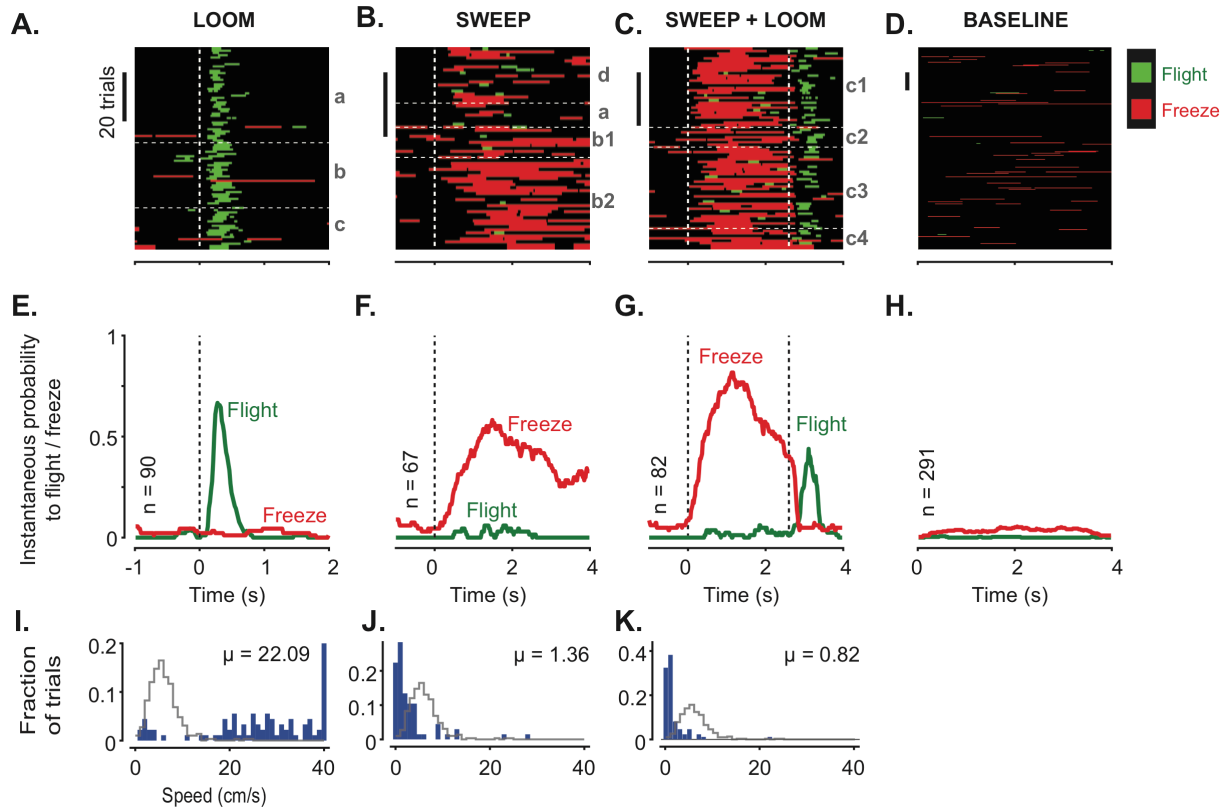
# B. SWEEP | LOOM



# C.

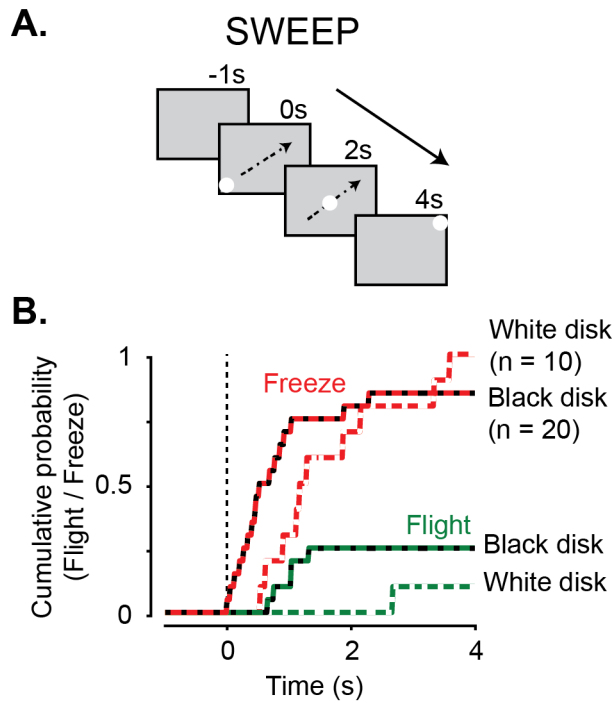


## Supplementary Information



**Figure S1 (related to Figure 1 and Figure 3):**

**A-D.** Ethograms. Movement speed was used to classify flight and freeze behaviours. Flights were defined as epochs where speed was greater than 40 cm/s, and return to refuge within 1 s. Freezes were defined as epochs in which speed decreased to less than 2 cm/s for at least 0.5 s. The vertical line indicates onset of the stimulus. The horizontal lines subdivide different cohorts of animals (see Experimental Procedures). **E-H.** Instantaneous probability of freeze and flight. The probability of observing a flight (green) or a freeze (red) at each time point. The dotted line indicates the onset of the stimulus. **I-K.** Average movement speed. Filled bars show distribution over trials of average movement speed during 0 s – 1 s after the loom stimulus onset (I), 0 s – 4 s after the sweep stimulus onset (J), or 0 s - 2.6 s after the sweep + loom stimulus onset; numbers show the mean across trials. The grey line indicates the distribution of average speed during baseline trials (which was mean 4.76 cm/s, s.d. 1.92).



**Figure S2 (related to Figure 2):**

**A.** Schematic of white sweep visual stimulus. The sweep stimulus was a 2.5 cm ( $5^\circ$ ) diameter white disc translating across the monitor at an angular speed of ca.  $21^\circ/\text{s}$  for 4 s.

**B.** The cumulative probability of having observed a flight (green-white) or freeze (red-white) response over time for a white sweep stimulus (10 trials in 10 animals). The cumulative probabilities of responses to a black sweep stimulus for the same cohort of animals are also shown (flight: green-black; freeze: red-black; 20 trials in 10 animals; replotted from Fig 2B).