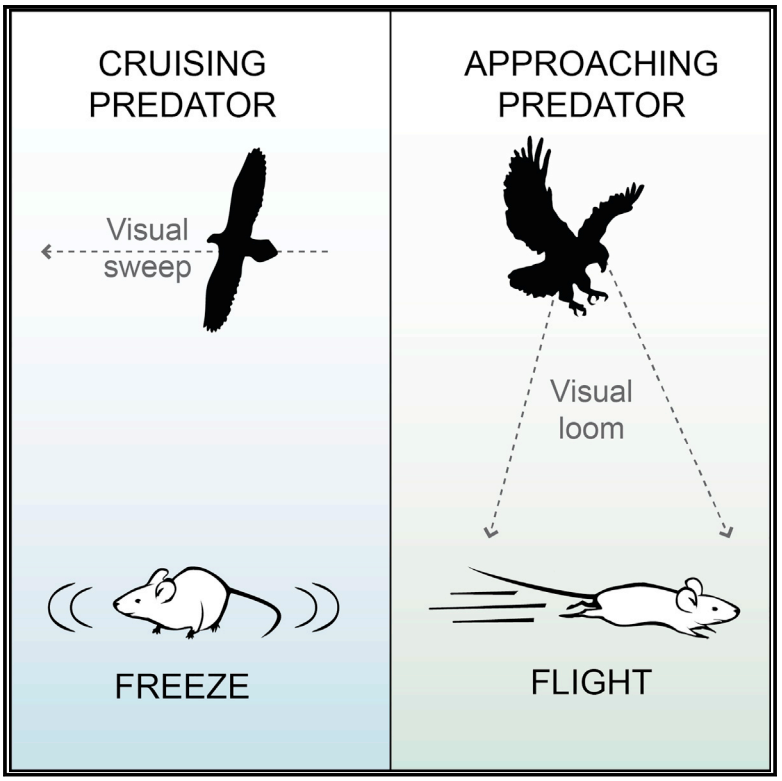
Report

Current Biology

Vision Guides Selection of Freeze or Flight Defense Strategies in Mice

Graphical Abstract



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In Brief

De Franceschi et al. discover that the visual simulation of a predator cruising overhead induces freezing responses in mice. Visual simulation of a rapidly approaching predator instead induces flight responses. These findings provide evidence that mice innately make behavioral choices based on vision alone.

Highlights

- Rodents use vision to choose how to respond to an overhead threat
- A moving disk induces freezing, while an expanding disk induces flight responses
- Opposing innate behaviors can be induced by visual stimuli

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Vision Guides Selection of Freeze or Flight Defense Strategies in Mice

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SUMMARY

In prey species such as mice, avoidance of predators is key to survival and drives instinctual behaviors 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 behaviors and provide evidence that mice innately make behavioral 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 characterized the behavior 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 gray screen. After habituating the mouse to the arena for 15 min, we triggered a visual stimulus when the mouse passed near the center 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 behavior, 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 s (Figure 1B). The stimulus emulates a 2 m wide predator, flying 25 m 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 nine of these, the animal froze before fleeing. Freezing behavior 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 behaviors, we analyzed the last 5 min of the habituation period (before any visual stimulus), analyzing only those epochs where the animal approached the center 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 behavioral response, and we therefore asked whether mice are sensitive to the speed of the sweep. In a new cohort of ten 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 behavior (Movie S2), occasionally with long-latency flight. Faster sweep stimuli (42 °/s; Figure 2C) led to freezing behavior, 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 behavior (latency 705 ± 163 ms, mean ± SEM; 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 was longer than those evoked by loom stimuli (218 ± 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

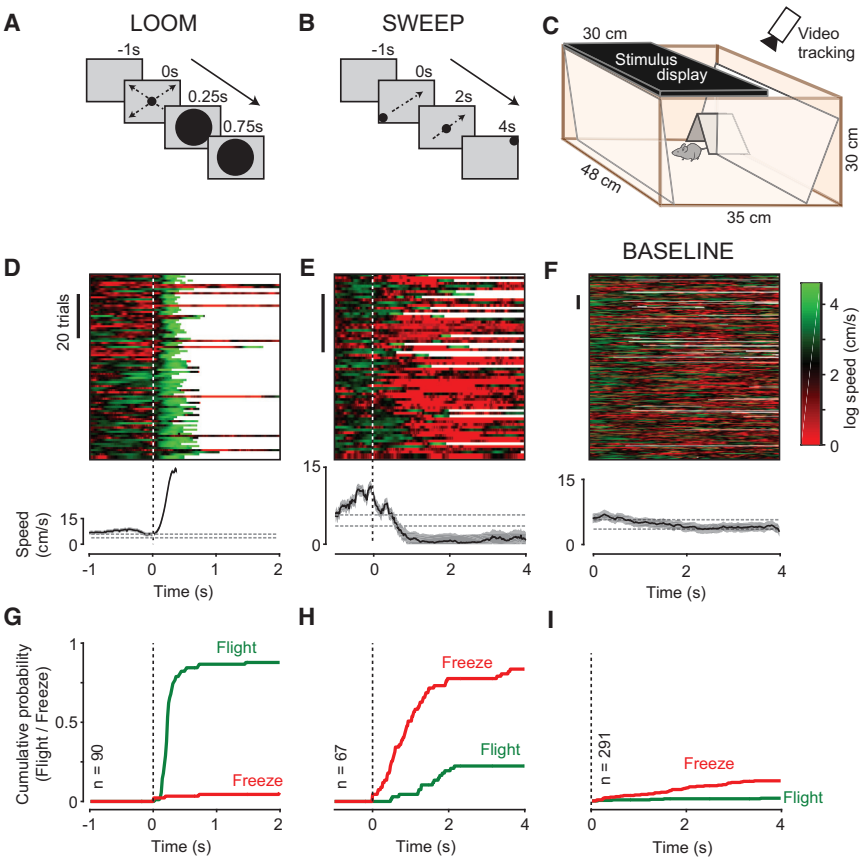

Figure 1. Visual Stimulus Dependence of Freeze and Flight Behaviors in Mouse

(A and B) Schematics of visual stimuli. The loom stimulus expanded from 1 to 25.5 cm (2°–50°) in 250 ms and persisted for 500 ms. The sweep stimulus was a 2.5 cm (5°) diameter black disk translating across the monitor at an angular speed of ca. 21 °/s for 4 s.

(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) Top: 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; white indicates times when the animal was in the refuge. Bottom: mean (±1 SEM) movement speed of mice across trials. Traces are clipped after flight home. Horizontal dashed lines indicate mean ± 1 SEM of movement speed in absence of visual stimuli, as shown in (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.

movement speed before flight commenced, but looms were not (Figure 2G; Movie S2).

Does freezing behavior 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%; Figures 3B and 3C) was similar to that in absence of a preceding sweep stimulus. Latency to flight after onset of loom stimulus was 250 ± 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 behaviors 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 behaviors, and a clear demonstration of the utility of vision for mice. Previous attempts to influence the choice of freeze and flight behaviors [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 behaviors might be mediated by distinct visual pathways. Specialized 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 behavioral response to loom stimuli [3, 14]. The sweep-induced behaviors that we observe might also be mediated by specialized subcortical pathways. For example, recent work shows a class of neurons in the mouse superior colliculus ("wide-field cells"), which respond to small moving stimuli over a large region of the visual field [15]. Cortical contributions to defensive behaviors are also likely, as visual cortical projections to superior colliculus in mouse both modulate visual responsiveness [16] and help drive temporary arrest behaviors [17].

Flight behavior can be rapid and reproducible following loom stimuli. However, the variable latency to flight during presentation of sweep stimuli (e.g., Figures 2A–2C), the direct path back to refuge, and the fact that flights are less likely when refuge is unavailable [8] suggest that flight behavior is not a simple reflex. Further, flight behaviors can be initiated even while freezing (e.g., Figure 2G). This suggests that during freezing behavior, mice are engaged in sustained assessment of their defense strategies, allowing deliberation and selection of an optimal strategy. Defining an optimal defense 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 behaviors through easily controlled visual stimuli. Combined with the availability of genetic tools in mice, this new framework

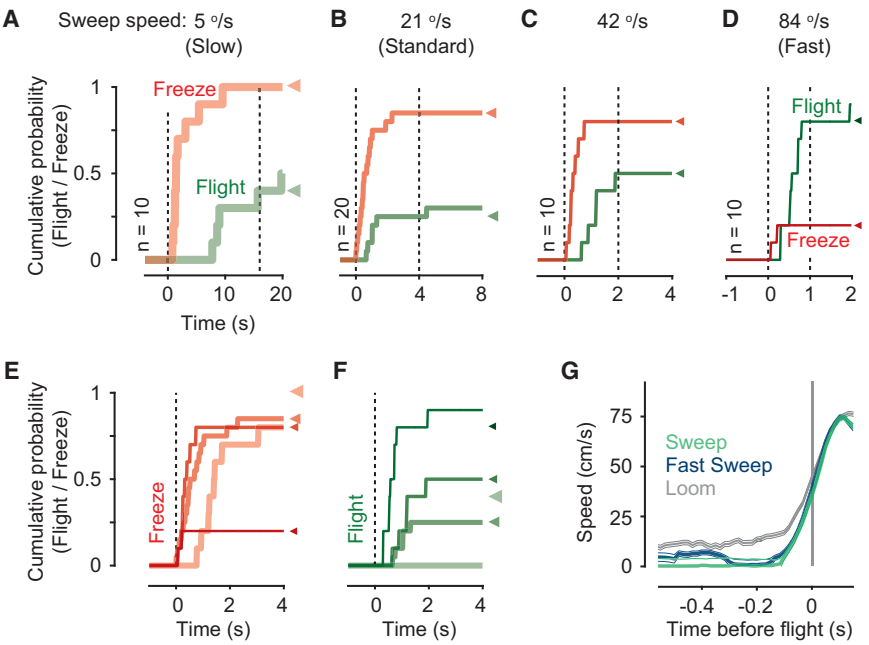

Figure 2. Dependence of Freeze and Flight Behaviors on Stimulus Speed

(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 °/s), over the first 4 s 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 (±1 SEM) of movement speed around the time of flight responses during presentation of standard sweep (21 °/s, n = 6 flights from 20 trials), fast sweep (84 °/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 Figure S2 and Movie S2.

may help us to 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 and Visual Stimulation

The behavioral arena was a 48 cm wide × 35 cm deep × 30 cm high box. An opaque triangular refuge 20 cm wide × 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 gray screen (48 cm × 27 cm, mean luminance 30–40 candela/m^2, 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) 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 s (21 °/s). In some experiments, the same black disk instead moved across the monitor in 16 s (5 °/s), 2 s (42 °/s), or 1 s (84 °/s) or was a white disk 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 2.6 s, 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 min; in subsequent trials, the habituation period was 5 min. After habituation, a visual stimulus was triggered when the animal's location was approximately under the center of the monitor. One trial was conducted each day, except in one cohort of animals (cohort a, defined below) where the loom stimulus followed the sweep stimulus by at least 1 min.

Cohorts

A total of 65 adult mice were housed under 12:12 light/dark cycle and tested during the dark period. Cohort a (Figures S1A and S1B) was eight male adult wild-type mice (C57BL/6, aged 13–18 weeks) and was tested once for the sweep stimulus and then six times for the loom. Cohort b (Figures S1A and S1B) was ten male adult wild-type mice (C57BL/6, aged 11–12 weeks) that were tested four times for the sweep stimulus (the first encounter is indicated by b1; subsequent encounters are indicated by b2) and then three times for the loom stimulus. Cohort c (Figure S1C) was 18 adult wild-type mice (C57BL/6, four female, aged 8–10 weeks), tested four (eight animals) or five (ten animals) times for the sweep + loom stimulus. Ten of the animals were also tested two times for the loom stimulus. In the sweep + loom trials, the looming disk expanded from either the final position of the sweep (cohort c3 and c4) or from an alternative location of the sweep trajectory (cohort c1 and c2). 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), six were adult wild-type mice (C57BL/6, aged 8 weeks with an exception of 43 weeks), and two were of other genetic profiles on C57BL/6 background (aged 7–9 weeks). Subdividing this cohort into animals aged 13 weeks or less (n = 14), aged more than 28 weeks (n = 5), or having the 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 ten 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 six sessions. The order of stimuli was randomized 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 center-of-mass of these pixels was used to define mouse position on each

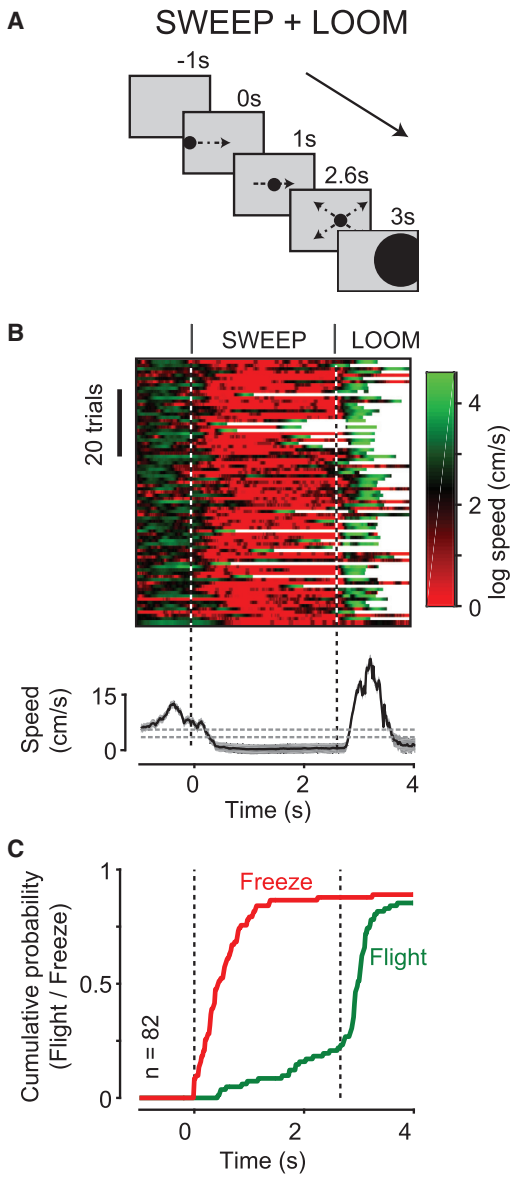

Figure 3. Behavioral Responses to Combinations of Sweep and Loom Stimuli

(A) Schematic of visual stimulus. The standard sweep stimulus (21 °/s) was presented for 2.6 s and was immediately followed by a loom stimulus.

(B) Top: 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. Bottom: mean (±1 SEM) movement speed of mice across trials. Horizontal dashed lines indicate mean ± 1 SEM 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.

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 s 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 s. Average speed across trials was calculated as the geometric mean and the SEM of the geometric mean. For baseline measurements, we analyzed activity prior to presentation of visual stimulus. We analyzed 4 s video sequences that were triggered on the animal moving away from the walls toward the center 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 20 cm/s above that at stimulus onset (response on one 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.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2016.06.006.

A video abstract is available at http://dx.doi.org/10.1016/j.cub.2016.06.006#mmc5.

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 behavioral monitoring software was written by G.D.F. and S.G.S. Experiments were conducted by G.D.F. and T.V., and data were analyzed by all authors. G.D.F., A.B.S., and S.G.S. wrote the paper.

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