Mice learn multi-step routes by memorizing subgoal locations

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The behavioral strategies that mammals use to learn multi-step routes are unknown. In this study, we investigated how mice navigate to shelter in response to threats when the direct path is blocked. Initially, they fled toward the shelter and negotiated obstacles using sensory cues. Within 20 min, they spontaneously adopted a subgoal strategy, initiating escapes by running directly to the obstacle’s edge. Mice continued to escape in this manner even after the obstacle had been removed, indicating use of spatial memory. However, standard models of spatial learning—habitual movement repetition and internal map building—did not explain how subgoal memories formed. Instead, mice used a hybrid approach: memorizing salient locations encountered during spontaneous ‘practice runs’ to the shelter. This strategy was also used during a geometrically identical food-seeking task. These results suggest that subgoal memorization is a fundamental strategy by which rodents learn efficient multi-step routes in new environments.

Escape behavior offers a powerful model for studying naturalistic navigation in the laboratory. Diverse animals, including fishes, lizards, crabs, birds and rodents, respond to threats by escaping to a familiar shelter. Mice are known to rapidly identify and memorize shelter locations in new environments and instinctively respond to visual or auditory threats by running straight to the shelter. Previous studies showed that the spatial memory for running back to shelter (‘homing’) can be based on path integration or distal visual landmarks when a direct path is available. If the direct path is blocked on one side by a barrier, previous work showed that gerbils can use spatial memory to reach the hidden shelter after a brief period of exploration. Thus, rodent escape offers not only reliable, stimulus-locked trajectories and rapid learning within a single session but also a reliance on spatial reasoning. These qualities make escape a useful model for understanding how animals learn and execute complex goal-directed trajectories within the time constraints compatible with survival in natural settings.

Here we first investigate the strategies that naive mice use to navigate to shelter in response to auditory threats when the direct path is blocked by a wall. Through quantitative analysis of escape trajectories and their relationship to exploratory behavior, systematic variation of spatial conditions and dynamic modifications to the environment, we describe how mice learn to execute efficient multi-step escape routes within minutes of entering a novel, obstacle-laden environment. We then show that the navigational strategy for escape is also used for reaching a food reward goal.

Results

Mice rapidly learn efficient escape routes past an obstacle. As a baseline condition for investigating how mice learn escape trajectories, we placed naive animals in a circular, open-field platform with a shelter and overhead lighting. After a brief exploration period during which mice spontaneously located the shelter, we exposed them to a loud, overhead crashing sound while they were in a pre-defined threat zone (Fig. 1a). This reliably elicited rapid escapes directed at the shelter along a straight ‘homing vector’ (n = 23 escapes, ten mice; Fig. 1a,b, Extended Data Fig. 1a and Supplementary Videos 1 and 2),
similar to previous results\(^7\). We then repeated this experiment in a separate group of mice, with a wall positioned between the threat zone and the shelter (\(n = 24\) mice; Extended Data Fig. 2a). This wall was white against a black background, and all mice approached and walked along it during the exploration period (Extended Data Fig. 1b). To quantify escape trajectories in relation to the obstacle,
we computed a target score: escapes initiated in the direction of the shelter get a score of zero; escapes targeting the obstacle edge get a score of 1.0; and escapes aimed beyond the obstacle edge get scores $>1.0$ (Fig. 1a). Escapes are classified as ‘edge vectors’ if their score surpasses the 95th percentile of escape scores in the open field (0.65) and are otherwise classified as ‘homing vectors’. Upon the first threat presentation, most mice (57%) executed homing vector escapes (Fig. 1a,b and Supplementary Video 3). Replacing the wall obstacle with an unprotected hole obstacle did not reduce this proportion (Extended Data Fig. 2b and Extended Data Fig. 1c,d). Thus, homing vector escapes cannot be purely accounted for by the safety provided by running along a wall; rather, they are likely directed at the shelter location.

Over the course of three threat presentation trials ($17 \pm 4$ min into the session, mean $\pm$ s.d.), mice performed escapes that were increasingly spatially efficient (ratio of the shortest possible path to the actual escape path: median for trial 1 $= 0.77$; for trial 3 $= 0.87$; $F_{2,30} = 7.2$, $P = 0.003$, repeated-measures analysis of variance (ANOVA) on trials 1–3; Fig. 1c) and rapid (normalized escape duration: median for trial 1 $= 3.8$ s; for trial 3 $= 3.2$ s; $F_{2,34} = 6.2$, $P = 0.005$; Extended Data Fig. 1e). By this point, almost all trajectories were aimed directly at the obstacle edge (90% edge vectors; median target score $= 0.98$). Thus, although inefficient homing responses initially dominated, mice acquired rapid and streamlined routes to shelter over the course of 20 min and three escape trials.

We next investigated whether mice use visual input to locate and run toward the obstacle edge. First, we tested whether visual cues are sufficient for generating edge vector escapes. After 20 min with three escape trials in the open field, we examined how mice responded to an unexpected obstacle rising up at the same time as the threat stimulus onset ($n = 10$ trials, ten mice; Extended Data Fig. 3a and Supplementary Video 4). We found that 7/10 escapes followed homing vector paths until reaching the wall (tactile obstacle avoidance: Extended Data Fig. 3a). The other 3/10 escapes deviated toward an obstacle edge before the mouse was close enough to touch the wall, suggesting that vision was used to navigate toward the obstacle edge (putative visual obstacle avoidance; proportion of mice deviating toward the edge: $3/10 > 0/2$ escapes in the open field; $P = 0.02$, Fisher’s exact test; Extended Data Fig. 3a). Thus, both tactile and visual cues can be used to negotiate obstacles during escape, in the absence of any prior experience with an obstacle. Second, we examined whether visual cues were necessary for generating edge vector escapes. We repeated the obstacle experiment from Fig. 1 but now in complete darkness (Extended Data Fig. 3b–d and Supplementary Video 5). Mice executed fewer edge vector escapes in the dark (% edge vector escapes on trials 1–3 was $33\%$ with the lights off versus $74\%$ with the lights on, $P = 0.002$, permutation test). This proportion of edge vector escapes was not significantly different than escapes in the open field (not more than the $22\%$ edge vector rate in the dark in the open field; $P = 0.2$, permutation test). However, after 20 min with three escape trials in the light, mice were able to execute mostly edge vectors in the dark (55% edge vector escapes versus $22\%$ in the open field, $P = 0.002$, permutation test; Extended Data Fig. 3c–d). Thus, for naive mice with limited experience, visual cues are required for efficient obstacle avoidance. However, immediately after experiencing a 20-min behavioral session in the light, streamlined escapes can occur even in complete darkness.

**Mice develop a spatial memory strategy for obstacle avoidance.** We, thus, considered that learning efficient escapes might entail developing a memory of the obstacle edge location, making perception of the obstacle unnecessary. To further test this hypothesis, after the animals explored the environment with the obstacle for 20 min and with three escape trials, we removed the obstacle at the moment of threat onset (‘acute obstacle removal’; Supplementary Video 6). Although the obstacle disappeared before the initial orientation movement could be completed, all animals escaped along the edge vector and did not turn toward the shelter until they passed the location where the obstacle edge used to be (median target $= 0.98$; $n = 8$ escapes, eight mice; Fig. 2a,b). Next, we examined how persistent this memory-based strategy is. In a ‘chronic obstacle removal’ experiment (CORE), we allowed several minutes between this acute obstacle removal trial and additional escape trials ($9 \pm 5$ min, mean $\pm$ s.d.). During this time, the mice explored the platform, including visiting its now obstacle-free center (Extended Data Fig. 4a). We found that 44% of the subsequent escapes were still directed at the location where the obstacle edge used to be ($n = 18$ escapes, eight mice; more than the $9\%$ edge vector rate in the open field: $P = 0.02$, permutation test; Fig. 2a,b and Supplementary Video 6), whereas the remaining $56\%$ of escapes reverted to the homing vector response.

This memory for edge vector escapes could, in principle, be learned during escape trials or through spontaneous exploratory behavior. To distinguish between these possibilities, we repeated the CORE but now with zero baseline escape trials during the initial 20-min exploration period (CORE-ZB). As in the previous experiment, we then removed the obstacle and allowed the mice to explore the newly unobstructed environment (for $5 \pm 4$ min, mean $\pm$ s.d.; Extended Data Fig. 4a). Threat presentation after this period resulted in mostly edge vector responses ($57\%$ edge vector escapes; $n = 23$ escapes, ten mice; more edge vectors than in the open field: $P = 0.004$, permutation test; Fig. 2a,b). Thus, within 20 min in a novel environment, mice spontaneously develop a persistent spatial memory for efficient, multi-step escapes.

Our experiments so far have revealed a memory-based obstacle avoidance strategy. We have also shown that visual input is used to navigate around the obstacle when experience is limited. We, therefore, tested how perception and spatial memory operate in tandem when both are fully available. In a novel environment, we performed an experiment similar to the CORE, but, instead of removing the obstacle, we changed its length by $25\%$ (Fig. 2c); obstacle shortened: $n = 14$ escapes, nine mice; obstacle lengthened: $n = 13$ escapes, nine mice; obstacle always short: $n = 15$ escapes, nine mice; obstacle always long: $n = 10$ escapes, eight mice). Initial escape trajectories were consistently biased toward the previous edge location (Fig. 2d,e, Extended Data Fig. 4b–e and Supplementary Video 7). However, this result differed from the obstacle removal experiments in two ways. First, the memory bias was intermediate in magnitude: escape targets were biased only partway toward the former edge location (Fig. 2d and Extended Data Fig. 4d,e). Second, the second segment of the escape was equally biased: after reaching the obstacle edge, mice ran toward where the shelter would be if the edge had not moved (Fig. 2e and Extended Data Fig. 4d–e). These results show that, when available, the current obstacle position is an important cue for anchoring and adjusting memory-guided paths to both the edge and the shelter.

**Characterizing the spatial memory strategy for escape past an obstacle.** We next aimed to characterize the spatial memory strategy and how it is learned. We evaluated three possible strategies: habitual learning of turn angles, sampling the environment to build a cognitive map and memorizing subgoals encountered during practice homings. We evaluated each possibility by analyzing the relationship between escapes and spontaneous behavior during exploration, primarily in the chronic obstacle removal experiment with zero baseline escape trials (CORE-ZB). For each analytical finding, we then performed additional experiments to validate the analysis.

**Habitual, egocentric movements do not explain the spatial memory: analysis.** First, we tested whether mice learn egocentric movements from the threat zone to the obstacle edge, similar to the habitual
response strategy in mazes\textsuperscript{6}. We extracted all spontaneous homing runs, defined as sustained turn-and-run movements from the threat area toward the shelter during the CORE-ZBs exploration period (Methods; median interquartile range (IQR) number of runs = 7 (6, 7); time from their end point until reaching the shelter = 8 (2, 16) s; Fig. 3a, Extended Data Fig. 5a and Supplementary Video 8). We then computed each run’s starting position and orientation and the angle turned during its initial turn-and-run segment (Fig. 3a; see Methods for details and Extended Data Fig. 6 for examples of starting point extraction).

Homing runs were sparse, and their initial positions and body orientations were highly variable. It was unlikely for any escape’s starting conditions to closely match a previous homing: only 22% of escapes were preceded by a run with starting points within 10-cm distance and 30° body orientation (Extended Data Fig. 5a). Despite this lack of stereotypy, we attempted to account for the memory-guided edge vector escapes observed in the CORE-ZB using the assumption that mice repeat turn angles from previous homing runs. First, we validated a method to predict escape targets based on homing run turn angles. We put mice on a modified

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**Fig. 2 | Mice use a spatial memory strategy for efficient obstacle avoidance.**

- **a.** Examples of edge vector escape trials (top) and all trajectories (bottom) after removing the obstacle. Subheaders describe the experience in the environment before removing the obstacle. The dotted line indicates where the obstacle used to be. Arrow plots show the distribution of initial escape targets. **b.** Summary data for initial escape targets after obstacle removal. One-sided permutation test: acute: ***P = 1 × 10^{-6}; chronic 3 baseline: *P = 0.02; chronic 0 baseline: **P = 0.004, permutation test on the rate of edge vectors compared to the open field. Acute removal: n = 8 escapes, eight mice; chronic removal, 3 baselines: n = 18 escapes, eight mice; chronic removal, 0 baselines: n = 23 escapes, ten mice. c. Schematic for the experiment in which the obstacle length is changed by 25%. For the ‘obstacle shortened’ condition, the dotted gray line indicates the initial obstacle length, and the thick black line indicates the new length (after 20 min/3 baseline escape trials). The initial and the shelter-bound segments of the escape are analyzed. d. Summary data for the experiments changing the length of the obstacle by 25%, analyzing the initial segment of the escape. Escapes aimed at the current obstacle edge location get a score of 0; escapes aimed at the edge location if the obstacle were 25% longer get a score of +1 (overshoot); and escapes aimed at the edge location if the obstacle were 25% shorter get a score of −1 (undershoot). Combining both length change experiments, the median of the initial target score’s absolute value is 0.44. One-sided permutation test on the initial escape target, obstacle shortened versus unchanged: P = 0.002 (**); obstacle lengthened versus unchanged: P = 0.002 (**). e. Summary data for the experiments changing the length of the obstacle by 25%, analyzing the shelter-bound segment of the escape. Escapes aimed at the current shelter location get a score of 0; escapes aimed at the shelter location if the obstacle were 25% longer get a score of +1 (overshoot); and escapes aimed at the shelter location if the shelter would be if the obstacle were 25% shorter get a score of −1 (undershoot). Combining both length change experiments, the absolute median shelter-bound-target score magnitude is 0.37. One-sided permutation test on the shelter-bound escape target, obstacle shortened versus unchanged: **P = 0.001; obstacle lengthened versus unchanged: ***P = 0.0002. Obstacle shortened: n = 14 escapes, nine mice; obstacle unchanged: n = 25 escapes, 17 mice; obstacle lengthened: n = 13 escapes, nine mice. White squares show the median; thick lines show the IQR; and thin lines show the range excluding outliers. Distributions are kernel density estimates.
Fig. 3 | Habitual, egocentric movements do not explain the spatial memory for escape. 

**a** Examples of homing runs—continuous turn-and-run movements toward the shelter—extracted from all movement data (Supplementary Video 9 and Methods). Turn angles are defined as the difference in heading direction between the homing initiation point and the point where the mouse has travelled 15 cm. Turn angles are positive for rightward turns and negative for leftward turns. **b** Predicting the escape targets of mice in the CORE-ZB on the basis of their previous turning movements. Left: diagram illustrating the method for predicting the escape target based on the turn angles from previous homing runs. Right: the y axis measures the amount of variance in escape targets across trials that can be explained by mice repeating turn angles from previous homing runs. The positive control is a platform with two narrow corridors, ensuring that homings and escapes are stereotyped (Extended Data Fig. 5; n = 30 escapes, ten mice). The test condition is the CORE-ZB. The negative control is predicting a random angle and then extrapolating that angle to predict escape targets in the CORE-ZB (average R² = 0.58 using the homing run with the closest initial body orientation; Fig. 3b and Extended Data Fig. 5c).

platform with two narrow corridors, ensuring that homings and escapes were stereotyped (n = 30 escapes, ten mice; Extended Data Fig. 5b). Here, we could precisely predict escape targets using the mouse’s starting point and its history of previous turn-and-run movements (R² of the prediction = 0.97 using the homing run with the most similar turn angle; R² = 0.65 using the homing run with the closest initial position; R² = 0.58 using the homing run with the closest initial body orientation; Fig. 3b and Extended Data Fig. 5c).
In the CORE-ZB, however, repeating turning movements did not explain any of the variance in post-removal escape targets ($R^2$ of the prediction $= 9 \times 10^{-4}$ (most similar turn angle); $R^2=0.04$ (closest initial position); $R^2=9 \times 10^{-6}$ (closest initial body orientation)); $R^2=0.05$ (randomly generated prediction); Fig. 3b and Extended Data Fig. 5d).

Habitual, egocentric movements do not explain the spatial memory: experiments. This analysis suggests that memory-guided edge vector escapes are not based on repeating egocentric actions that mice had executed during the exploration period. We performed two new experiments to test this finding. First, we performed a variant of the CORE-ZB with an additional barrier in front of the threat zone, which was removed simultaneously with the main obstacle (Fig. 3c; n=20 escapes, ten mice). With this extra barrier in place, homing runs now consisted of movements with smaller turn angles than in the previous experiments (the two COREs with the original geometry; $P=0.001$, chi-square test on the distribution of homing run turn angles; Fig. 3c). However, after obstacle removal, the distribution of escape turn angles was indistinguishable from the previous experiments ($P=0.6$, chi-square test on the distribution of escape turn angles; Fig. 3c). Thus, different fine-grained movements during exploration do not necessarily produce different escape movements. Also note that escape routes did not deviate around the second, distal obstacle location (Fig. 3c). This further suggests that memory-guided escapes are not merely a function of the movements made during exploration but, instead, depend on the geometry of the environment. In a second new variant of the CORE-ZB, we tested whether memory-guided escapes are sensitive to the shelter location, as this would not be expected from a habitually repeated action (Fig. 3d; n=18 escapes, ten mice). After the 20-min exploration period, we simultaneously removed the obstacle and moved the shelter to the middle of the platform. As expected from a goal-directed or geometry-dependent process, escape turns differed from the two original COREs, with zero escapes targeting the obstacle edge location ($P=0.5$, chi-square test on homing run turn angles; $P=0.03$, chi-square test on escape turn angles) (Fig. 3d). Both of these experiments demonstrate a dissociation between egocentric turning movements and memory-guided escapes: distinct exploratory movements can lead to identical escape movements, and identical exploratory movements can lead to distinct escape movements.

Mice memorize subgoals at previously targeted locations: analysis. The goal-directed nature of these escapes suggests that the obstacle edges become subgoal locations. An alternative possibility, however, is that mice target the edge by learning allocentric heading directions. For example, edge vector escapes could be generated by consistently running in the southwest or southeast direction, relative to the north–south axis connecting the shelter and the threat zone. Analysis of our data indicates that mice, instead, target allocentric locations. After obstacle removal, escape heading directions follow whichever direction is required to reach the edge location (correlation between the heading direction to the edge and the heading direction taken in the escape: $r=0.85$, $P=1.2 \times 10^{-4}$; Extended Data Fig. 5e). This corroborates the results above, suggesting that mice learn true subgoals at the obstacle edge.

We next investigated the learning process that generates these subgoals during the spontaneous exploration period. We found two variables in the CORE-ZB with high, positive correlations to subgoal-targeting behavior: the total distance of exploratory movement on the threat side of the platform (correlation with post-removal escape targets: $r=0.72$, $P=1 \times 10^{-4}$; Extended Data Fig. 7a) and the number of homing runs from the threat area that directly targeted the obstacle edge (within 10 cm; correlation with post-removal escape targets: $r=0.75$, $P=5 \times 10^{-5}$; Fig. 4a–c). Two primary interpretations of these correlations are possible. The first is that routes are computed directly from a ‘cognitive map’: investigating the obstructed area updates the mouse's internal map, which is reflected behaviorally in the mouse's use of subgoals. If this were true, we would predict that (1) investigating relevant features, such as the obstacle or its edge, will also correlate with the subgoal memory; and (2) after obstacle removal, investigating the region where the obstacle used to be will suppress edge vector escapes. Neither prediction matched the data. The amount of exploration near the obstacle or the obstacle edge was not correlated to subsequent escape target scores (correlation with distance moved around the obstacle: $r=-0.09$, $P=0.7$; with distance moved around the obstacle edge: $r=0.06$, $P=0.8$; Extended Data Fig. 7a). Furthermore, after obstacle removal, mice that densely sampled the empty center of the arena more did not execute different escape trajectories from mice that explored very little (correlation with distance moved around where the obstacle used to be: $r=-0.12$, $P=0.6$; with total post-removal exploration distance: $r=-0.17$, $P=0.4$; Extended Data Fig. 7a; see Extended Data Fig. 4a for examples of exploration during this period).

A second possibility is that learning occurs during the ‘practice’ edge vector homings. In this case, we would predict that (1) subgoals do not form in mice with zero edge vector homings, and (2) the correlation with spontaneous homing runs would be specific to the edge targeted during escape (that is, left versus right) and to the direction taken during escapes (that is, from the threat side to the shelter side). Both predictions were confirmed by the data. Every edge vector escape after obstacle removal was preceded by at least one homing run targeting that same edge (100% of post-removal edge vector escapes have ≥1 prior edge vector run; greater than chance: $P=0.02$, permutation test; Fig. 4c). Second, escape targets in the CORE-ZB were not significantly correlated with homing runs from the threat area to the opposite edge ($r=0.15$, $P=0.5$), with homing vector runs from the threat area to the middle of the obstacle ($r=0.06$, $P=0.8$), or with runs from the shelter area to the same obstacle edge ($r=0.30$, $P=0.2$) (Fig. 4a).

Mice memorize subgoals at previously targeted locations: experiments. Our analysis of the CORE-ZB suggests that executing edge vector homings, rather than sampling the environment, could be the rate-limiting step in spontaneously learning subgoals. To further test this hypothesis, we first examined whether spontaneous homings explain escape routes in the obstacle present condition. On the first trial with an obstacle, mice with prior edge vector homings performed more efficient escapes than mice with none (median spatial efficiency with zero runs = 0.76; with one run = 0.82; $P=0.04$, permutation test; Fig. 4d; same data from Fig. 1). As expected, this effect was specific to runs from the threat area to the side of the obstacle used during the escape (Extended Data Fig. 7b–d). Thus, subgoal memorization does appear to play an adaptive role when perception of the obstacle is still available. Next, we examined the acute obstacle removal experiment. We could not apply correlational analysis to the acute obstacle removal after three trials because this dataset had 100% edge vector responses and 100% prior edge vector homings. Thus, we performed a new experiment, removing the obstacle acutely on the first trial (10 ± 1 min into the session, mean ± s.d.). Here, 50% of escapes took edge vector paths (n=10 escapes, ten mice; Extended Data Fig. 7e). Among the variables examined—that is, exploration in different parts of the platform and various running movements—only the number of runs from the threat area to the edge used in the escape was significantly correlated with escape targets ($r=0.71$, $P=0.02$; Fig. 4e and Extended Data Fig. 7fg). Furthermore, 100% of edge vector escapes were preceded by at least one edge vector homing (greater than chance: $P=0.02$, permutation test; Fig. 4e).

Next, we tested the practice homing hypothesis with two new experiments. First, we repeated the CORE-ZB but without a shelter during the exploration period (n=24 escapes, ten mice; Fig. 4f).
Fig. 4 | Mice memorize previously targeted subgoal locations. a. Correlation of different running movements with the escape target score, in the CORE-ZB. These include homing runs from the threat area to different parts of the obstacle as well as runs from the shelter area to the obstacle edge. Movements toward the same edge targeted in the escape (here, the right edge) are considered separately from movements toward the opposite edge (here, the left). Boxes show the correlation coefficients and respective $P$ values; significant correlations have green outlines. b. Homing run history for two mice in the CORE-ZB and subsequent escape trajectories. c. Escape targets plotted against the number of spontaneous edge vector homing runs during exploration in the CORE-ZB. As shown in a, these only include movements toward the same edge targeted during the escape. d. Spatial efficiency of escapes on the first trial in the presence of an obstacle (same data as in Fig. 1). As in previous panels, edge vectors only include movements to the edge targeted during the escape. Zero edge vectors: $n=9$ escapes, 9 mice; $\geq 1$ edge vector: $n=12$ escapes, 12 mice. Two-sided permutation test: $^*P=0.04$. e. Escape targets plotted against the number of spontaneous edge vector homing runs during exploration, for acute obstacle removal on the first trial. f. Chronic obstacle removal experiment without a shelter present during the 20-min exploration period. All subsequent escapes are shown on the right. g. Chronic obstacle removal experiment with an extra barrier blocking the threat area during the 20-min exploration period. All subsequent escapes are shown on the right. h. Summary of escape targets in the two modified CORE-ZB experiments. One-sided permutation test on the proportion of edge vector escapes, compared to the open-field condition (as in Fig. 2b). No shelter condition: $n=24$ escapes, ten mice; block threat side condition: $n=25$ escapes, ten mice. White squares show the median; thick lines show the IQR; and thin lines show the range excluding outliers. Distributions are kernel density estimates.
This gives the mouse opportunity to observe the platform and obstacle but without performing homings. After 20 min, we added the shelter and removed the obstacle as soon as the mouse entered the shelter (median (IQR) time to enter shelter: 84 (39, 154) s). Subsequent escapes did not exhibit the subgoal memory (13% edge vector escapes; not more edge vectors than in the open field: \( P = 0.4 \), permutation test; Fig. 4f,h). Second, we repeated the CORE-ZB with an extra barrier blocking off the threat side of the platform during the exploration period (\( n = 25 \) escapes, ten mice; Fig. 4g). This prevents long-range homings while allowing investigation of the obstacle. Only 1/10 mice targeted the edge location with scores close to 1.0, and post-removal escapes did not significantly differ from the open-field control (20% edge vector escapes; not more edge vectors than in the open field: \( P = 0.2 \), permutation test; Fig. 4g,h). Both experiments, thus, demonstrated that merely investigating the obstacle does not lead to memorizing subgoals. They, therefore, lend support to the hypothesis that subgoal locations are, instead, learned through practice homings.

**Spontaneous edge vector runs are instinctive exploratory actions.** It remains unclear what prompts spontaneous edge vector homings in the first place. One possibility is that, during practice homings, a cognitive map is used to compute efficient routes to shelter; this could allow mice to target a subgoal for the first time, at which point that location could be memORIZED for use in subsequent escapes. Another possibility is that mice are innately predisposed to run to visually salient obstacle edges. Our data support the latter option. Spontaneous edge-directed movement occurs most during the first few minutes of the session and occurs equally with or without a shelter in the environment (Extended Data Fig. 8a,b). When the obstacle is a hole instead of a wall (Extended Data Fig. 2b), edge-directed movement is much less frequent, occurring at the same rate as in the open field (computed relative to the location where the obstacle edges would be if the obstacle were present; Extended Data Fig. 8b). Correspondingly, it takes twice as long for mice to learn to perform predominantly edge vector escapes in the presence of a hole obstacle (20% edge vector escapes on trials 2–3, 67% edge vector escapes on trials 6–7; Extended Data Fig. 8c,d).

**Subgoal learning also supports food-seeking routes.** Although subgoal memorization enhances spatial efficiency in a static environment, it also generates unnecessarily roundabout routes past an obstacle that no longer exists. In fact, edge vector escapes can persist over at least 20 min and seven trials after obstacle removal (Extended Data Fig. 9a,b). We considered that subgoal memorization might be specific to escape behavior, as mice might sacrifice flexibility for the sake of quickly reacting to imminent threats. To test this, we performed an obstacle removal experiment in the context of a less urgent, reward-based task (open-field control: \( n = 32 \) reward runs, six mice; obstacle removal: \( n = 34 \) reward runs, six mice). First, we trained food-deprived mice to approach and lick a reward port in response to a 10-kHz tone, which indicated the availability of condensed milk at the port. This took place across five sessions, in an operant conditioning box (Extended Data Fig. 10a–c). Next, we transported this task to the platforms previously used for escape behavior. The shelter was replaced by the reward port, and the threat stimulus was replaced by the 10-kHz tone (Fig. 5a). To start the session, mice were given 20 min in the obstructed environment (or in the open-field control environment) with \( \pm 1 \) baseline food approach trial per minute. Next, we removed the obstacle (or simply continued the session in the open-field condition), triggered food approach trials (5 ± 3 min after obstacle removal, mean ± s.d.) and recorded the resulting food-seeking routes (Fig. 5a). At this point, mice successfully ran to the reward port during tone presentation on 85% of trials but with slower reaction times than escape (median (IQR) time to start running toward the goal for food seeking = 1.5 (0.7, 3.5) s; for threat response = 0.6 (0.4, 1.2) s; \( P = 0.005 \), permutation test). Similarly to escape routes, a large proportion of paths in the obstacle removal condition initially targeted the obstacle edge location (53% edge vectors; \( P = 0.006 \) compared to 12% in the open-field control session, permutation test; Fig. 5a,b and Supplementary Video 9; see Supplementary Fig. 2 for correlations of food-seeking target with time, trial and exploratory behavior).

Finally, we tested whether experience with the obstacle induces a non-specific increase in edge-directed movement, as this could explain the apparent use of subgoal memorization across two distinct tasks. We compared spontaneous movements from the ends of the platform toward the center and obstacle edge locations. Exploration after obstacle removal was not enriched in edge-directed movements (number of edge-directed movements per 15 min: median with the obstacle present = 10; after obstacle removal = 3; in the open field = 4; \( P = 2 \times 10^{-3} \), permutation test on open field versus obstacle; \( P = 0.9 \), permutation test on open field versus obstacle removed) (Fig. 5c,d). Subgoal memorization, therefore, reflects a strategy for goal-directed navigation rather than a general bias in how mice move around their environment after experience with an obstacle.

**Discussion**

During their first few minutes in an obstructed environment, mice escaped to shelter by relying on their memory of the shelter location and their innate ability to negotiate barriers using vision and touch. These escape routes were spatially inefficient; they resembled obstacle avoidance in animals with lower cognitive capacities, such as toads, crabs and ant colonies. After a single 20-min session, however, mice began to exploit their aptitude for spatial memory. They increasingly targeted the obstacle edge directly and could do so even in complete darkness or after the obstacle had been removed. We found that this capacity relied on memorizing allocentric subgoal locations rather than egocentric turning movements, and our data further suggest that mice identified and memorized subgoals during spontaneous homing runs.

Previous work showed that rodents use spatial memory to navigate to shelter in an open field. In such a simple environment, however, escape routes can be implemented by path-integrating self-motion cues to keep track of a single vector to the shelter location—a one-step, egocentric process. With obstacles in the environment, a more advanced strategy is needed. Previous results in gerbils escaping in an obstructed environment suggested that spatial memory was employed to reach the shelter, but their navigational strategy was unknown. Our results show that mice use subgoals in an allocentric reference frame. Several observations support this view. First, mice can accurately target the edge location minutes after the obstacle or the lights have been removed, which is not well explained by pure path integration. Second, escapes involved immediately orienting and running toward a subgoal ~50 cm away, which is not consistent with following odor trails or gradients. Finally, repeating stereotyped turning movements or allocentric heading directions did not explain memory-guided escape paths in our assay; instead, mice consistently targeted the edge location. Future experiments on how escape routes transfer across days, types of obstacle, spatial scales and tasks will help to specify the nature of this allocentric schema and how it is updated.

Traditional models of allocentric navigation involve three key elements: an internal map of the environment (located in the hippocampus and entorhinal cortex), a stored goal location and a mental search for paths to the goal. The limiting factor is the quality of the map. Finding efficient multi-step routes—be it through a tree-search algorithm, a map-partitioning algorithm or warping around an ‘obstacle-to-avoid’ feature—can occur as soon as the map faithfully reflects the current environment. To build up this map, animals simply have to investigate unfamiliar or altered parts...
of the environment. The amount of exploratory movement matters for spatial learning, but movements’ intentions or directions do not\(^{29}\) (compare to ref. \(^{28}\)). Our observations of escape routes in naïve mice do not support such views of allocentric learning. In our data, none of the following was sufficient to generate subgoals: (1) spending time exploring the obstacle; (2) running along the homing vector path and then being blocked by the obstacle; (3) learning a subgoal at the other obstacle edge; (4) targeting the obstacle edge while running away from the shelter; (5) investigating the obstacle in the absence of a shelter; and (6) investigating the obstacle while the threat area was blocked off. Furthermore, investigating the formerly obstructed area after obstacle removal did not restore direct homing vector responses.

The subgoal strategy does contain elements of classical map-based navigation: it is learned in all-or-none fashion and depends on a sense of allocentric space—that is, a ‘map’; however, it also includes a component similar to taxon navigation, in which animals learn inflexible routes based on previous goal-directed movements\(^{11}\). Hybrid strategies—combining rapid learning, inflexible routes and special ‘learning movements’—have been discovered before, as in the orientation flights of wasps\(^{10}\). However, orientation flights entrain one-step routes to a visual beacon; here, we found that learning movements are used to entrain multi-step routes to an obstructed goal. Our working model is that mice instinctively execute visually guided movements toward a salient wall edge; if this movement gives the mouse direct access to a subsequent goal (for example, the shelter), then its target is memorized as a subgoal location. We hypothesize that a rapid, all-or-none learning rule works on practice homings, but further experiments should be done to test the causal role of this putative moment of insight.

Memorizing subgoals confers distinct survival advantages: it can drive escape routes with the optimality of map-based planning and the rapidity of instinctive responses. However, this strategy is less flexible than responding to sensation or updating maps. The steady persistence of ~50% biphasic escapes for tens of minutes after removing the obstacle was longer than expected, and it remains unclear how mice learn to reinstate the homing vector response after obstacle removal. Responses to imminent predatory threats are known to favor quick reaction times at the expense of computational sophistication\(^{31}\), and so this inflexible strategy could, in principle, be specific to defensive behavior. However, we found that it was also used in a less urgent food-seeking task. Thus, subgoals appear to be a general building block for quickly learning spatial locations important for survival.

**Fig. 5** | Experience with an obstacle changes food-seeking but not exploratory trajectories. a. Food approach paths in response to a 10-kHz tone associated with the availability of condensed milk at a reward port. An example trial is shown on top, and all paths are shown below. The red circle with ‘R’ represents the reward location—that is, the metal spout with milk. The dotted line indicates the location of the obstacle during the initial 20-min period. b. Summary data for food-seeking paths, computed the same way as escape targets. One-sided permutation test on the proportion of edge vector paths: \(**P = 0.006\). Open field: \(n = 32\) reward runs, six mice; obstacle removed: \(n = 34\) reward runs, six mice. c. Paths across the platform during spontaneous exploration in the escape experiments. All paths go from the ends of the platform toward the center. Conditions with more sessions are randomly downsampled so that the same number of paths is displayed for each condition. d. The number of spontaneous center-directed and edge-directed movements during exploration. One-sided permutation test on the number of edge-directed movements; obstacle versus open field: \(***P = 2 \times 10^{-5}\); obstacle removed versus open field: \(P = 0.9\). Obstacle: \(n = 24\) mice; obstacle removed (CORE with zero and with three baseline trials): \(n = 20\) mice; open field: \(n = 10\) mice. White squares show the median; thick lines show the IQR; and thin lines show the range excluding outliers. Distributions are kernel density estimates. NS, not significant.
Subgoal learning bears some resemblance to hierarchical reinforcement learning, a technique in artificial intelligence for learning multi-step behaviors\textsuperscript{17,18}. However, the learning process we have observed in mice does not fit cleanly into the dominant ‘model-free versus model-based’ framework for reinforcement learning agents (compare to ref. \textsuperscript{19}). Rather, it fuses action repetition with a model of space: mice discover a map of individual subgoals through targeted exploration and learning heuristics.

Finally, our results provide an alternative entry point to studying the neural mechanisms of spatial learning. Experiments with constrained behaviors and open-field environments have been crucial for the spatial memory field; they uncovered the hippocampal formation's key role in allocentric spatial memory\textsuperscript{20,21}, identified important activity dynamics in the hippocampus\textsuperscript{22–25} and demonstrated the dorsal striatum's involvement in repeating inflexible routes\textsuperscript{26,27}. However, models of navigation based on these data remain limited in their applicability to real-world learning. To improve on this, future work will benefit from an understanding of spontaneous learning strategies and complex behaviors\textsuperscript{28–30}. Probing brain activity during spontaneous subgoal memorization presents one such opportunity for reconciling neural models with natural learning. This behavior's rapid learning profile and reliable, stimulus-locked routes make it particularly tractable for testing theories of hippocampal and striatal functions and of the neural dynamics that underlie them.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41593-021-00884-8.

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Methods

Animals. All experiments were performed under the UK Animals (Scientific Procedures) Act of 1986 (PPL 70/7652) after local ethical approval by the Sainsbury Wellcome Centre Animal Welfare Ethical Review Body. We used 158 singly housed (for ≥2 days/week), male, 9–13-month-old C57BL/6J mice (Charles River Laboratories) during the light phase of the 12-h light/dark cycle. Mice were housed at 22 °C and in 55% relative humidity with ad libitum access to food and water. For the main experiments, data come from the mice’s first-ever behavioral session. Table 1 describes all groups of mice used for this study, including the experimental conditions, any excluded mice and re-use across experiments.

Behavioral platforms. The main platform was an elevated white acrylic circular platform 92 cm in diameter (Extended Data Fig. 2a). The platform had a 51 cm × 1 cm hole in its center; through this hole, the obstacle (white acrylic, 50 cm long × 12.5 cm tall × 0.5 cm thick) could be raised (obstacle condition) or lowered (open field condition). For experiments in which the obstacle disappears or the obstacle was done by digitally triggering a custom-made pneumatic tubing system (time to raise or lower the obstacle was ~100 ms; Supplementary Video 10). In the acute obstacle removal experiment, this was triggered simultaneously with the stimulus onset. In chronic obstacle removal experiments, this was triggered while the mouse was in the shelter. Obstacle removal makes a ‘whooshing sound’ (63 dB measured in the shelter) and usually triggers a startle response. The presence or absence of this response was unrelated to subsequent expression or extinction of the subgoal memory. The hole obstacle consisted of a 50-cm-long × 10-cm-wide rectangular hole in the center of the platform (Extended Data Fig. 2b). The modified platform with two narrow corridors consisted of the original platform with the obstacle and a plus six additional panels (schematic in Extended Data Fig. 5b). Four of these panels were 50 cm long × 12.5 cm tall × 0.5 cm thick, and two were 12.5 cm long × 12.5 cm tall × 0.5 cm thick. Together, they formed two corridors that were 50 cm long × 7.5 cm wide and were at 65° and 115° angles relative to the axis of the central obstacle. The interior panels forming the corridor were made of red acrylic so that the infrared camera could see through them; all other panels were made of white acrylic. The platform in the sideways-moving obstacle experiment was an elevated white acrylic square platform 80×80×80 cm. The obstacle (white acrylic, 70 cm long × 12.5 cm high × 0.5 cm thick) was manually pulled to the ‘short’ condition (obstructing 48 cm) or to the ‘long’ condition (obstructing 66 cm) while the mouse was in the shelter. The shelter was a 10-cm cube of transparent red acrylic (opaque to the mouse). It included a mouse-hole-shaped entrance at the front and additional 2.5-cm-tall square of red acrylic on top to prevent the mice from climbing on top. The platform was surrounded by a black square plastic surrounding. A projector screen was located above the platform. The platform was illuminated with four infrared lights (S8100-45-A-IR, Fuloon). Experiments shown in Extended Data Fig. 3 and Supplementary Video 5 were performed in complete darkness (0.00 cd m⁻² of visible light). At this light level, mice did not react to rapidly waving a hand in front of them, which is perceived as highly threatening when light is available. For all other experiments, light was projected onto the screen at 5.2 cd m⁻² using a projector (PF1000U, LG). Note that the room was not totally dark and partially illuminated and that neither the black surround nor the overhead illumination was circularly symmetric; these asymmetries could all provide spatial orientation cues. The platform and shelter were cleaned with 70% ethanol after each session.

Escape behavior. Animals were given a 7-min acclimation period during which they discovered the shelter. Stimulation was subsequently delivered when the mouse entered the threat zone (the back 20 cm, on the end opposite from the shelter) and was generally facing away from the shelter. Only stimuli delivered in this zone were analyzed. At least 1 min was allowed in between trials. Stimulation stimuli were loud (87 dB), unexpected crashing sounds played from a speaker located 1 m above the center of the platform (Supplementary Audio 1 and 2). Sounds (‘smashing’ and ‘cracking fireplace’) were downloaded from soundbible.com. They were then edited using Audacity 2.3.0, such that they were 1.5 s long and continuously loud. Stimulation stimuli were alternated between the ‘smashing’ sound and the ‘cracking’ sound to prevent stimulus habituation. In some sessions (four with and four without an obstacle), we used an ultrasonic sweep stimulus (3 kHz to 60 kHz, 3 s) presented in response to the stimulus and observed, and, therefore, the data from these sessions were pooled. For each trial, the stimulation was triggered repeatedly until the mouse reached the shelter, for a maximum of 9 s. Because escapes take longer with the hole obstacle and in the dark, stimuli in these conditions were played for up to 12 s. Stimulus responses were considered escapes if the mouse reached the shelter within 12 s in the light or 18 s for the hole obstacle and for the dark. To distinguish escape behavior from exploration-like routes, the rare trials in which, after initiating the escape, the mouse moved back toward the threat zone on average over a 2-s period were not counted as escapes.

Stimulus delivery was controlled with software custom written in LabVIEW (2015 64-bit, National Instruments). Stimuli were triggered manually, when the mouse had been in the threat zone (demarcated on the live video) for at least 1 s and was facing in approximately the opposite direction from the shelter. Although manual stimulation could be a source of bias, we found that mice executing homing vector versus edge vector escapes did not have significantly different starting positions or heading directions, limiting the effect that this bias could have (Supplementary Fig. 1a–c). We also found that the obstacle removal experiments with differing results did not exhibit significantly different starting positions or orientations (Supplementary Fig. 1d). The signal was compared to 30-Hz pulse with video frame acquisition, which was also fed as an input to the input/output board and sampled at 10 kHz. To verify correct synchronization, in most experiments the audio output cable was also fed in parallel to an infrared LED (850 nm OSLON PowerStar IR LED), which flashed in sync with sound presentation. Mice varied in how many trials they performed in each experiment, due either to remaining in the shelter rather than entering the threat zone or not escaping in the desired time frame (Supplementary Fig. 1e). We thus limited analysis to the first three escapes in each condition (more than 50% of mice completed at least three escapes in all experiments).

Food-seeking behavior. Mice were food restricted to 85% of their baseline weight. Training and pre-training were done in a 60 cm × 15 cm rectangular arena, with a shelter on one side and a reward port on the other side. The reward consisted of a 7-μl drop of condensed milk (diluted 1:1 with water) delivered through the spout. For pre-training, during which the mouse learned to associate the metal spout with reward, 100 drops of milk were manually triggered and then collected by the mouse, with a minimum interval of 1 min between each drop. They were then trained with each test session – 1-min extinction to approach and latch a metal spout in response to a 9-s, 10-kHz, 72-dB tone. Tone stimuli were triggered manually once per minute. Licking the spout while the tone was on resulted in reward. After reward delivery, there was a 5-s refractory period; thus, mice could trigger, at most, two rewards during the 9-s tone. On the last two days of training, the tone duration was reduced to 4.5 s after 30 min. Licks were registered with a capacitive touch sensor (Adafruit MP121), connected to a microcontroller board (Arduino Uno). The milk was delivered through a peristaltic pump (Campden Instruments, 80204E), connected to the same microcontroller. For testing food-seeking paths, these mice had two sessions. The first session was in the platform with no obstacle, the shelter on one side and a lick port on the opposite end of the platform. They received ‘practice trials’ of tone and milk, from throughout the platform but initially mostly when they were already near the lick port. After 20 min, test trials were initiated when the mouse was on the opposite side from the lick port, and these data were used for analysis. The second session followed the same protocol. However, in this session, the obstacle was initially present and then was removed after 20 min while the mouse was in the shelter. Mice performed more trials than with the escape behavior, so here we examined trajectories from the first eight successful trials (50% of mice completed at least eight trials).

Video tracking and visualization. Videos were acquired at 30 frames per second using an overhead camera (AcA1300-60GM NIR, Basler) with a near-infrared-sensitive (NIR) video recording camera and software custom written in LabVIEW (2015 64-bit, National Instruments). Videos were then filtered and post-processed. The video was then analyzed using custom Python code using the OpenCV library41 (Supplementary Video 1). We used DeepLabCut3 to track the mouse from the video, after labeling 1,500 frames with 13 body parts: snout, left eye, right eye, left ear, neck, right ear, upper limb, upper right limb, lower limb, lower right limb, lower back, left upper limb, lower back, right upper limb, left upper limb, and tail. Post-processing included removing low-confidence tracking, using a median filter with a width of 7 frames and applying an affine transformation to the tracked coordinates to match the common coordinate framework.

Analysis. All analysis was done using custom software written in Python 3.6 as well as open-source libraries, notably OpenCV and scikit-learn41,43.

Calculating position, speed and allocentric heading direction (Supplementary Video 11). For analysis of trajectories and exploration, we used the center of the mouse. This was calculated as the average of all 13 points, which we found to be more stable and consistent than using any individual point. To determine the mouse’s speed for the color-coded visualizations, we smoothed the raw frame-by-frame speed with a Gaussian filter (sigma = 3 frames = 0.1 s). To calculate the mouse’s heading direction, we computed the vector between the center of the body (averaging the tail base, right hind limb, lower back, left hind limb, right upper limb, upper back and left upper limb) and the front of the body (averaging the left upper limb, upper back and right upper limb points). We set the south direction (threat to shelter) to 0°, north (shelter to threat) at 180° and east/west (right/left sides) as ±90°. Quantification of escape targets. The initial escape target was computed by taking the vector from the mouse’s position at the escape initiation to its position when it is 10 cm in front of the obstacle. For the hole obstacle, this means 10 cm in front of the obstacle’s outer perimeter rather than its center. We computed a target score where a vector aimed directly at the shelter received a value of 0; one aimed at...
either obstacle edge received a value of 1.0; a vector halfway between these would score 0.5; and a vector that points beyond the edge would receive a value greater than 1.0. The formula is:

\[ \text{score} = \frac{\text{offsetEV}}{\text{offsetHV}} \]

where \( \text{offsetEV} \) is the distance from the mouse to where the mouse would be if it took the homing vector; \( \text{offsetHV} \) is the distance from the mouse to where the mouse would be if it took the obstacle edge vector; and \( \text{offsetHV} \) is the distance from the homing vector path to the obstacle edge vector path. Only the obstacle edge closest to the escape path was considered. Initial food approach trajectories and spontaneous exploration trajectories were analyzed in the same manner. The threshold for classifying a trajectory as an edge vector was the 95th percentile of escapes in the escape path.

Table 1 | All groups of mice and all experimental conditions

| Environment | Light | # of mice | Excluded—no threat zone entry | Previous stimulus experience | Time before trial 1 | Data location |
|-------------|-------|-----------|-------------------------------|-----------------------------|---------------------|--------------|
| Open field  | On    | 10        | 0                             | None                        | 10 min              | F1, EDF 2, F5, EDF 10 |
| Obstacle    | On    | 24        | 0                             | None                        | 10 min              | F1, EDF 2, F4, EDF 6, F5, EDF 10 |
| Hole obstacle | On    | 8         | 0                             | None                        | 10 min              | EDF 2, EDF 7 |
| Obstacle rises acutely | On | 10 | 5 | 3 trials (Open field) | 20 min | EDF 3 |
| Obstacle rises acutely | On | 9 | 4 | 1 session + 3 trials (Open field) | 20 min | EDF 3 |
| Open field | Dark  | 14        | 0                             | None                        | 10 min              | EDF 3 |
| Obstacle    | Dark  | 14        | 0                             | None                        | 10 min              | EDF 3 |
| Obstacle    | 10 min on, then dark | 14 | 0 | None | 10 min | EDF 3 |
| Obstacle    | 20 min on, then dark | 14 | 0 | 3 trials (Obstacle) | 20 min | EDF 3 |
| Acute obstacle removal trial 4 | On | 10 | 2 | 3 trials (Obstacle) | 20 min | F2 |
| Chronic obstacle removal (CORE 1 (CORE-3B)) | On | 10 | 2 | 3 trials (Obstacle) + 1 trial (Acute removal) | 20 min | F2, F4A, EDF 8, F5, EDF 10 |
| CORE 2 (zero baseline escape trials; CORE-ZB) | On | 10 | 0 | None | 20 min | F2, F3, EDF 5, F4, EDF 6, EDF 8, F5, EDF 10 |
| Square with short obstacle | On | 12 | 3 | 1 session | 10 min | F2, EDF 4 |
| Square with long obstacle | On | 12 | 4 | 1 session | 10 min | F2, EDF 4 |
| Square with lengthened obstacle | On | 9 | 0 | 1 session + 3 trials (long obstacle) | 20 min | F2, EDF 4 |
| Square with shortened obstacle | On | 12 | 3 | 1 session + 3 trials (short obstacle) | 20 min | F2, EDF 4 |
| Narrow corridors | On | 10 | 0 | 1 session | 20 min | F3, EDF 5 |
| CORE 3 (additional short barrier) | On | 10 | 0 | None | 20 min | F3 |
| CORE 4 (move the shelter) | On | 10 | 0 | None | 20 min | F3 |
| Acute obstacle removal trial 1 | On | 10 | 0 | None | 10 min | F4, EDF 6 |
| CORE 5 (no shelter at first) | On | 10 | 0 | None | 20 min | F4, EDF 7 |
| CORE 6 (additional long barrier) | On | 10 | 0 | None | 20 min | F4 |
| Open field (no shelter) | On | 6 | 0 | 1 session | n/a | EDF 7 |
| Obstacle (no shelter) | On | 6 | 0 | 1 session | n/a | EDF 7 |
| Hole obstacle (no shelter) | On | 7 | 0 | 1 session | n/a | EDF 7 |
| Open field (food seeking) | On | 6 | 0 | 5 training sessions (conditioning box) | 20 min | F5, EDF 9, EDF 10 |
| CORE 7 (food seeking) | On | 6 | 0 | training + 1 session (open field food) | 20 min | F5, EDF 9, EDF 10 |

Table 1: All groups of mice and all experimental conditions. Mice that were excluded generally stayed inside of the shelter instead of exploring. This is more common in mice with more previous experience. Previous stimulus experience refers to previous trials and sessions from an experiment higher up on the list. For example, ‘3 trials (Obstacle)’ comes from the ‘Obstacle’ condition listed higher up in the table.
to outside of the threat zone after a threat stimulus. This is computed in the same way for spontaneous homings. In practice, the escape initiation point occurs when the mouse starts turning to run along the path that leads it out of the threat zone. We use this criterion because it allows us to fairly compare spontaneous and stimulus-evoked homings; it correctly rejects initial post-stimulus movement bouts directed away from the shelter; and it correctly identifies the beginning of a turn-and-run movement as verified by manual inspection of the videos. Illustration of the escape initiation points for the main experiments is displayed in Extended Data Fig. 6. To characterize the urgency of the task based in the escape task versus in the food task, we use the metric of the time until running toward the goal. This is when the mice cross a threshold on the homing speed of $\geq 20$ cm s$^{-1}$.

**Quantification of turning angles.** Turning angles that initiated homing runs and escapes were taken as the difference between the mouse's heading direction at the start of the movement (the homing run or escape initiation point) and the mouse's heading direction after it had traveled 15 cm away from this start location. The start location is when the mouse starts turning toward and/or moving toward the shelter or obstacle edge (see previous subsection). Left turns were defined as negative; right turns were defined as positive. For predicting escape targets from turn movements, we first extracted all homing runs from the mouse's previous exploration experience. We then identified the homing runs(s) most similar to the escape, using three different similarity metrics: the most similar turn angle, the closest starting position and the closest initial heading direction. For each homing run–escape pair, we computed what the escape target would have been if the mouse had turned the same angle that it had turned during the homing run—that is, if it had repeated the previous egocentric action. Finally, we performed a linear regression between the predicted targets ($\hat{y}$) and the actual escape targets ($y$) to find the proportion of variance ($R^2$) in escape targets predicted using this assumption that mice repeat previous egocentric turns. $R^2 = 1 - \frac{\sum (y_i - \bar{y})^2}{\sum (y_i - \bar{y})^2}$, where $\bar{y}$ is the mean escape target, and $\bar{y}$ is the predicted escape target based on the linear regression. For the negative control, we disregarded the homing experience and, instead, predicted a random turn angle and then extrapolated that angle to predict an escape target. We repeated this procedure 1,000 times to get 1,000 $R^2$ values and took the mean $R^2$.

**Quantification of exploration.** The time spent exploring was computed as the time spent at least 5 cm away from the shelter. The amount of exploration, or distance explored, was the time exploring multiplied by the mouse's speed at each time point. Mice spent 1–3 of the session in the shelter (IQR: 20–52% of the time). Spontaneous exploratory traversals are paths during exploration that start at either end of the platform (within 20 cm the end) and then reach within 10 cm of the central x axis. Traversals that go along the boundary of the platform (that is, within 10 cm of the outer perimeter) or take longer than 2 s (10 cm s$^{-1}$) were excluded from analysis, as these paths contained pausing and looping behavior, hindering the analysis of trajectories.

**Statistics.** For permutation tests, the test statistic is the group mean difference (for example, in escape target or path efficiency). The condition of each mouse (for example, open field versus obstacle) is randomly shuffled 10,000 times to generate a null distribution and a $P$ value. We used this test because it combines two distinct advantages. (1) Because the test statistic is the group mean, this test gives weight to each trial that a mouse performs rather than collapsing each animal's data into one mean or improperly pooling trials (unlike the t-test or the Mann–Whitney test). (2) It is non-parametric and does not assume Gaussian noise (unlike the repeated-measures ANOVA), in line with much of our data. Tests for differences in efficiency, reaction time and initial escape conditions were two-tailed; tests for targets being biased toward previous experience were one-tailed. A different test statistic was used for the permutation test testing the significance of the result that 100% of memory-guided edge vector escapes had at least one prior edge vector homing. In this test, the $P$ value reflects the proportion of random subgroups of all trials that also score 100%. For the CORE-ZB (23 escapes, 13 edge vector escapes), the $P$ value of 0.02 indicates a 2% chance that every member of a random subgroup of 13/23 escapes has $\geq 1$ prior edge vector movement. The ANOVA was performed using the linear mixed-effects model package in R, after removing outliers ($z$-score $> 0.975$). The normality assumption was tested using the Shapiro–Wilk test. Fisher's exact test was performed for cases with one trial per mouse and a categorical outcome. The Pearson correlation coefficient was used for correlation analyses. $R^2$ values report the percent of the variance explained by the two variables' linear relationship and is equivalent to the square of their Pearson correlation coefficient. Sample sizes for initial experiments were not predetermined but were chosen to be similar to previous publications14,15,17,18. Subsequently, a power analysis was carried out based on estimated means and variances across conditions and a minimum power of 0.8, determining sample sizes of ten animals in the light and 14 in the dark. The ranges illustrated in box plots are limited from the first quartile minus 1.5x IQR to the third quartile plus 1.5x IQR. Statistically significant results are indicated in the figures using *$P<0.05$, **$P<0.01$ and ***$P<0.001$.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Data supporting the findings of this study (mouse tracking data and stimulus information) are available from https://figshare.com/articles/dataset/Subgoal_paper_data_zip/16461035. Any additional data are available from the corresponding author upon reasonable request.

**Code availability**

The custom code used to perform analysis for this study is available at https://github.com/BrancoLab/escape-analysis; code for registering videos to a common coordinate framework is available at https://github.com/BrancoLab/Common-Coordinate-Behaviour; and code for creating the visualizations found in the figures and supplementary videos is available at https://github.com/BrancoLab/escape-visualization.

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**Author contributions**

P.S. and T.B. conceived the project, designed the experiments and wrote the manuscript. P.S. performed the escape experiments with help from P.I. P.S. analyzed the data. D.C. and N.B. designed the food reward training protocol. S.F.O. and P.S. performed the food reward experiments.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41593-021-00884-8.

**Supplementary information**

The online version contains supplementary material available at https://doi.org/10.1038/s41593-021-00884-8.

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**Peer review information**

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Extended Data Fig. 1 | Escapes in the presence of an obstacle. a, Speed profile for all escape trials (3 trials per mouse). Mice were stimulated with sound (dotted white line) while in the threat zone of the open field (N = 10 mice; left) or the platform with the obstacle (N = 24 mice; right). In the platform with the wall obstacle, the probability of threat-evoked escape to shelter was 93%, while the probability of escape in a no-stimulus control was 12%. Trials are sorted by shelter arrival time. b, Exploration trajectories around the obstacle area (each color represents the movements of one mouse prior to trial 1). 3 randomly selected sessions are displayed. All mice approached and explored the area within 5 cm of the obstacle prior to the first escape trial (median time in this region: 37 sec, IQR: 29 - 45 seconds, minimum 14 seconds; median total distance explored in this region: 417 cm, IQR 259 - 493 cm, minimum 127 cm). c, Example trial and escape trajectories for experiment with an unprotective hole obstacle instead of the wall obstacle (N = 23 escapes, 8 mice). Grey indicates homing-vector paths, and blue indicates obstacle-edge vector paths. The black rectangle represents the hole obstacle. d, Summary of escape trajectories with the wall and hole obstacles. e, Escape duration measured as the duration from escape initiation until reaching the shelter. To compare across conditions and trials, this value is normalized by the shortest possible path length to the shelter from the mouse’s starting point. Each dot represents one escape. f, Running speed during escape is the average speed of the mouse from escape initiation until reaching the shelter. There is a small effect of trial on running speed. F(2, 37)=4.4, P = .02, two-sided repeated measures ANOVA on trials 1-3. Open field total: N = 23 escapes, 10 mice; obstacle trial 1: N = 21 escapes, 21 mice; obstacle trial 2: N = 24 escapes, 24 mice; obstacle trial 3: N = 21 escapes, 21 mice.
Extended Data Fig. 2 | Platforms with the wall obstacle and hole obstacle. a, Picture of the platform with the wall obstacle. The platform is 92 cm in diameter, and the wall obstacle is 50 cm long x 12.5 cm tall. The shelter is 10 cm long x 10 cm wide x 12.5 cm tall. It is made from red acrylic that is opaque to the mouse but transparent to red and infrared light. b, Picture of the platform with the hole obstacle. The platform is 92 cm in diameter and has a 50 cm long x 10 cm wide x 1 m deep rectangular hole in the middle. Both platforms are raised from the floor at a height of 1 m.
Extended Data Fig. 3 | The role of visual input in efficient obstacle avoidance. 

**a.** Example trial and all escape trajectories when the obstacle arises simultaneous with stimulus onset. This trial occurs after 20 minutes with three baseline escape trials in the open field. Putative visual avoidance occurs when the mouse turns toward an obstacle edge in the region between 5-10 cm away from the obstacle. Putative tactile obstacle avoidance occurs when the mouse turns toward an obstacle edge only after its head is already within 5 cm of the obstacle. N = 10 escapes, 10 mice. 

**b.** Escape trajectories for experiments in which naïve mice escape to the shelter in complete darkness (the four experiments in panels B and C are the only experiments in this paper performed in the dark). Dot-and-arrow plots display the distribution of escape targets in each condition. Open field: N = 41 escapes, 14 mice; obstacle: N = 33 escapes, 14 mice; obstacle + exploration in light: N = 33 escapes, 14 mice. 

**c.** Mice with 20 minutes of experience in the light, including three escape trials. Even after removing all light, mice execute edge-vector responses. N = 32 escapes, 14 mice. 

**d.** Summary of escape trajectories in complete darkness. One-sided permutation test on edge vectors in the dark, obstacle vs. open field; after 20 mins in the light: P = 0.002 (**); after 10 mins in the light: P = 0.2; exploration in dark: P = 0.2.
Extended Data Fig. 4 | Obstacle manipulation experiments. a, Chronic obstacle removal experiments (combined data from the experiment with three baseline escape trials and the experiment with zero). After the obstacle had been removed and prior to the escape trials, 100% of mice had previously visited the area where the obstacle used to be (black bar). Each colored trace represents the movements of one mouse after the obstacle was removed and prior to an escape trial. Six mice were randomly selected for visualization. b, Escape trajectories in a control condition for the obstacle length-change experiment. In this condition, the obstacle is always short. N = 15 escapes, 9 mice. Grey indicates trajectories targeting the shorter obstacle edge location, and blue indicates trajectories targeting the longer obstacle edge location. Arrow plots: dark green indicates overshooting the current edge or shelter position, and light green indicates undershooting (see Fig. 2c). c, Escape trajectories in a control condition for the obstacle length-change experiment. In this condition, the obstacle is always long. N = 10 escapes, 8 mice. d, Escape trajectories in an obstacle length-change experiment. The obstacle starts out long (dotted line) and is shortened after 20 minutes and 3 escape trials. N = 14 escapes, 9 mice. e, Escape trajectories in an obstacle length-change experiment. The obstacle starts out short (dotted line) and is lengthened after 20 minutes and 3 escape trials. N = 13 escapes, 9 mice.
Extended Data Fig. 5 | Homing runs, turn angles, and heading directions. a. Left: histogram of homing runs’ initial condition. This shows, for an average escape in the CORE-ZB, how many prior homing runs fell into different proximity bins. Each bin reflects proximity in both the position (x-axis) and body orientation (y-axis) of the homing’s starting point. Right: example of homing runs extracted from exploration during the CORE-ZB’s exploration period, and example of a subsequent escape in that experiment. b. Experiment with narrow corridors that constrain movements during exploration and escape. Histogram and homing runs are computed in the same manner as in panel a. c. Correlation between the predicted escape target (using the procedure illustrated in Fig. 3b) and the actual escape target. Here, escape targets are predicted using the homing run with the most similar turn angle to the escape turn angle. Data are from homings and escapes in the platform with narrow corridor. The correlation coefficient r = 0.98; P = 2x10^-22. This prediction thus yields an R^2 value of 0.97, which here corresponds to a mean absolute error of 0.025 in escape-target units. d. Same analysis as in panel c, but with escapes and homings from the CORE-ZB. The correlation coefficient r = -0.03; P = 0.9. This prediction thus yields an R^2 value of 0.0009, which corresponds to a mean absolute error of 0.38 in escape-target units. For comparison, predicting that every escape will be equal to the CORE-ZB’s mean escape target generates a mean absolute error of 0.31. e. Correlation between the allocentric heading direction required to target the obstacle edge location (x-axis) and the allocentric heading direction during escape (y-axis). The y-axis heading direction is measured when the mouse is 15 cm away from the escape initiation point (that is, after the initial turn movement is complete). The vector from the center of the platform to the shelter (pointing south) is set as 0°, and a vector pointing west or east is ±90°. The absolute value of the heading direction is taken so that escapes toward the left and right edges can be considered together. Homing-vector escapes are not included. Data are from the two COREs of Fig. 2a,b. Lines show the linear regression fit, and the shaded area shows the prediction interval within 1 standard deviation.
Extended Data Fig. 6 | Illustration of escape initiation points in the main experiments. a, Escapes in the open field. The escape initiation points mark the beginning of a turn-and-run movement from inside the threat zone (gray area) to outside the threat zone. This is computed the same way for escapes (shown here) and for spontaneous homing runs (for example Fig. 3, Extended Data Fig. 5). Top left: red dots show the mouse’s position when the stimulus comes on for all trials, and blue dots show the mouse’s position at the escape initiation point. Bottom left: single escape trials are color-coded by trajectory type (homing-vector paths are black/gray, edge-vector paths are blue). Movements between the stimulus onset and the escape movement onset are shown in dark, bold traces. The rest of the escape is shown in a lighter hue. Right: example escape. The red mouse silhouettes mark the path between stimulus onset and escape initiation. The bright blue silhouette marks the escape initiation point, which is where the analysis of escape paths and turn angles begins. b, Escapes with an obstacle (trial 1). c, Escapes with an obstacle (trial 2). d, Escapes with an obstacle (trial 3). e, Escapes after chronic obstacle removal (zero baseline escape trials). f, Escapes after chronic obstacle removal (three baseline escape trials). g, Food-seeking trials in the open field. h, Food-seeking trials after chronic obstacle removal.
Extended Data Fig. 7 | Mice memorize previously targeted subgoal locations. **a**, Correlation between escape targets in the CORE-ZB and the amount of exploration in different sections of the platform. Red outlines indicate the section of the platform in which the distance explored is measured. For exploration near the obstacle edge, only the edge that was targeted during the escape (that is, left vs. right) is considered. Boxes show the correlation coefficients and respective p-values; significant correlations have green outlines. **b**, Spatial efficiency of escapes on the first trial in the presence of an obstacle (same data as in Fig. 1). Here, runs from the threat area to the 10 cm in the center of the obstacle are considered. Zero homing vectors: N = 9 escapes, 9 mice; ≥1 homing vector: N = 12 escapes, 12 mice. White squares show the median, thick lines show the IQr, and thin lines show the range excluding outliers. Two-sided permutation test on escape efficiency. **c**, Same as panel **b**, but here, runs from the threat area to the obstacle edge that was not used during the escape are considered. Zero edge vectors: N = 8 escapes; ≥1 edge vector: N = 13 escapes. **d**, Same as panel **b** and **c**, but for runs from the shelter area to the obstacle edge used during the escape. Zero edge vectors: N = 2 escapes; ≥1 edge vector: N = 19 escapes. **e**, Escapes from an experiment acutely removing the obstacle on the first trial, after 10 minutes of exploration. N = 10 escapes, 10 mice. **f**, Correlation of different running movements to escape target score, in the trial-1 acute removal experiment. These include homing runs from the threat area to different parts of the obstacle, as well as runs from the shelter area to the obstacle edge. Runs toward the same edge targeted in the escape (here, the right edge) are considered separately from runs toward the opposite edge (here, the left). **g**, Correlation between escape targets in the trial-1 acute removal experiment and the amount of exploration in different sections of the platform. Post-removal exploration is not applicable in this experiment, since the obstacle is removed just before the escape begins.
Extended Data Fig. 8 | Edge-directed movements in different environments. a, Frequency of spontaneous movements toward the obstacle edges (all sessions with an obstacle). b, Frequency of edge-directed movements for different conditions. Two-sided permutation test on number of edge-directed movements; wall obstacle vs. open field: \( P = 0.0005 \). Open field (no shelter): \( N = 6 \) mice; hole obstacle (no shelter): \( N = 7 \) mice; wall obstacle (no shelter): \( N = 16 \) mice. For movements directed toward the center of the platform, there are no significant differences across conditions. Each dot is one session. White squares show the median, thick lines show the IQR, and thin lines show the range excluding outliers. c, Escape trajectories for a hole obstacle (\( N = 53 \) escapes, 8 mice). The black rectangle represents the hole obstacle. d, Evolution of escape targets for increasing trial numbers with the hole obstacle and the wall obstacle.
Extended Data Fig. 9 | Edge vectors persist over many trials and minutes after obstacle removal. a, Escape targets vs. trial number in the chronic obstacle removal experiments (three and zero baseline escape trials combined, and now including the minority of mice that performed >3 trials). For this plot, only successful escape trials are counted toward the trial number. The correlation between escape target and the number of stimulus-evoked escapes following obstacle removal is not significant: correlation coefficient $r = -0.07$, $P = 0.6$. b, Escape targets vs. time. The correlation between escape target and amount of time since obstacle removal is not significant: correlation coefficient $r = -0.12$, $P = 0.3$. See Extended Data Fig. 6 for correlations between escape targets and the amount of post-removal exploration in various parts of the platform. Lines show the linear regression fit, and the shaded area shows the prediction interval within 1 standard deviation.
Extended Data Fig. 10 | Training mice to approach and lick a spout in response to a tone. 

**a.** Lick raster plots for an example mouse during the first (top) and the last training day (bottom). During food-approach training, a 9-second, 10-kHz tone is associated with the availability of condensed milk at a metal spout. For the lick raster plots, licks were plotted at 5 licks/sec when the sensor was tonically triggered by licking; this does not affect the quantifications in panels **b–c.** **b.** Summary data for lick probability during training. Relative lick probability is the average probability of licking the spout within a 4.5-second window during the stimulus, divided by the lick probability during the 20 seconds before or after the stimulus. Mice lick the spout specifically in response to the tone on the fifth day of training (relative lick probability > 1, \( p = 0.002, \) one-sided permutation test) but not on the first day (\( p = 0.09 \)). **c.** Summary data for reward-port approach probability during training. Relative approach probability is the average probability of moving from the back of the conditioning box to the side where the spout is located in response to the tone, divided by the approach probability at other random time points during the session. Mice approach reward specifically in response to the tone on the fifth day of training (relative approach probability > 1, \( p = 0.02, \) one-sided permutation test) but not on the first day (\( p = 0.41 \)). For panels **b** and **c,** gray lines are individual mice and the green line is the mean. \( N = 5 \) mice.
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☐ Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
Commercial software used in this study: LabVIEW (2015 64-bit, National Instruments); open source software: Audacity 2.3.0

Data analysis
Plotting and analysis in Python 3.6 (code available at github.com/BrancoLab/escape-analysis); Open source Python packages: OpenCV, scikit-learn, DeepLabCut; some statistical analysis in R. Custom software was not central to the paper.

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Data supporting the findings of this study (mouse tracking data and stimulus information) are available from figshare.com: figshare.com/articles/dataset/Subgoal_paper_data_zip/14610135. Any additional data are available from the corresponding author upon request.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample sizes for initial experiments were not predetermined but were chosen to be similar to previous publications. Subsequently, a power analysis was carried out based on estimated means and variances across conditions and a minimum power of 0.8, determining sample sizes of 10 animals in the light and 14 in the dark. |
| Data exclusions | As stated in the methods (Table 1), mice were excluded from analysis if they did not enter the threat zone during the experiment. |
| Replication | Main findings were reproduced across several differing experiments. Specifically, the subgoal memory phenomenon was repeated in geometrically identical conditions across three independent groups of mice and was replicated in each case. |
| Randomization | Animals in test and control groups were litter mates and randomly selected. |
| Blinding | Behavioral experiments were not performed blind to condition, as the experimenter who applied the threat stimuli could not do this without also setting up and monitoring the experimental setup. Behavioral data was analyzed applying the same automated criteria to all conditions. |

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Materials & experimental systems

| n/a | Involved in the study |
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| n/a | Involved in the study |
| ☒ | ChIP-seq |
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| ☒ | MRI-based neuroimaging |

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | We used singly housed (for ≥1 week), male, 8-12-week-old C57BL/6J mice (Charles Rivers) during the light phase of the 12-h light-dark cycle. Mice were housed at 22°C and 55% relative humidity with ad libitum access to food and water. |
| Wild animals | The study did not involve wild animals |
| Field-collected samples | The study did not involve samples collected from the fields |
| Ethics oversight | All experiments were performed under the UK Animals (Scientific Procedures) Act of 1986 (PPL 70/7652) following local ethical approval by the Sainsbury Wellcome Centre Animal Welfare Ethical Review Body. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.