LETTER

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Visual place learning in *Drosophila melanogaster*

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The ability of insects to learn and navigate to specific locations in the environment has fascinated naturalists for decades. The impressive navigational abilities of ants, bees, wasps and other insects demonstrate that insects are capable of visual place learning[1–4], but little is known about the underlying neural circuits that mediate these behaviours. *Drosophila melanogaster* (common fruit fly) is a powerful model organism for dissecting the neural circuitry underlying complex behaviours, from sensory perception to learning and memory. *Drosophila* can identify and remember visual features such as size, colour and contour orientation[5,6]. However, the extent to which they use vision to recall specific locations remains unclear. Here we describe a visual place learning platform and demonstrate that *Drosophila* are capable of forming and retaining visual place memories to guide selective navigation. By targeted genetic silencing of small subsets of cells in the *Drosophila* brain, we show that neurons in the ellipsoid body, but not in the mushroom bodies, are necessary for visual place learning. Together, these studies reveal distinct neuroanatomical substrates for spatial versus non-spatial learning, and establish *Drosophila* as a powerful model for the study of spatial memories.

Vision provides the richest source of information about the external world, and most seeing organisms devote enormous neural resources to visual processing. In addition to visual reflexes, many animals use visual features to recall specific routes and locations, such as the placement of a nest or food source. When leaving the nest, bees perform structured 'orientation flights' to learn visual landmarks. If subsequently displaced from their outbound flight, bees take direct paths back to their nests using these learned visual cues[7]. However, it is not clear how insects, which have relatively compact nervous systems, perform these navigational feats. In mammals, the identification of place, grid and head direction cells suggests the existence of a 'cognitive map'[8]. Unfortunately, little is known about the cellular basis of invertebrate visual place learning. To identify the neurons and dissect the circuits that underlie navigation, we studied place learning in a genetically tractable model organism, *Drosophila melanogaster*.

To test explicitly for visual place learning in *Drosophila*, we developed a thermal–visual arena inspired by the Morris water maze[9] and a heat maze used with cockroaches and crickets[1,2] (Fig. 1a). In the *Drosophila* place learning assay, flies must find a hidden 'safe' target (that is, a cool tile) in an otherwise unappealing warm environment

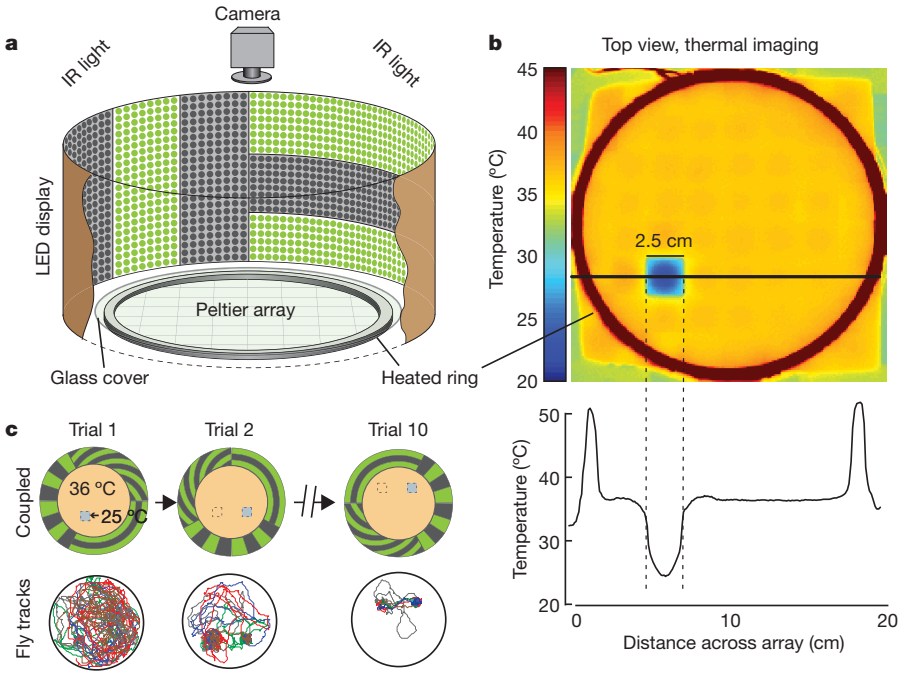

Figure 1 | *Drosophila* trained in a thermal–visual arena show place learning. **a**, Illustration of the arena. The floor is composed of 64 thermoelectric modules (a Peltier array), the panorama is provided by a 24 × 192 light-emitting-diode (LED) display and flies are recorded using a camera under infrared (IR) illumination. **b**, Top: thermal imaging view of the arena's floor showing the uniformly warm surface with a single cool tile; also shown is the heated ring barrier. Bottom: temperature readings across the arena. **c**, Trajectories of four representative flies from trials 1, 2 and 10 are shown below a diagrammatic representation of the visual panorama denoting the locations of the cool tile in the previous trial (dashed square) and in the current trial (blue square). In this coupled condition, the position of the cool tile relative to the visual panorama remains constant even as its absolute position changes between trials.

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(36 °C; Fig. 1b). Notably, there are no local cues that identify the cool tile. Rather, the only available spatial cues are provided by the surrounding electronic panorama that displays a pattern of evenly spaced bars in three orientations (Fig. 1a, c). To assay spatial navigation and visual place memory, fifteen adult flies are introduced in the arena and confined to the array surface by placing a glass disk on top of a 3-mm-high aluminium ring. During the first 5-min trial, nearly all flies (94%) eventually succeed in locating the cool target (data not shown). In subsequent trials, the cool tile and the corresponding visual panorama are rapidly shifted to a new location (rotated by either 90° clockwise or 90° anticlockwise, chosen at random). Importantly, the target and visual panorama are coupled so that although the absolute position of the cool tile changes, its location relative to the visual panorama remains constant (Fig. 1c). Our results (Fig. 2a, red trace, and Supplementary Movie 1) demonstrate that over the course of ten training trials flies improve dramatically in the time they require to locate the cool tile. This improvement is accomplished by taking a shorter (Fig. 2b), more direct route to the target (Fig. 2c), without noteworthy changes in the mean walking speed (Fig. 2d). To ensure that social interactions between flies were not influencing place learning (for example flies following each other to the safe spot), we also trained single flies and found that flies tested individually show equivalent place learning (Supplementary Fig. 1). As would be predicted for bona fide visual place learning, the improvement in place memory is critically dependent on the visual panorama. Flies tested in the dark show no improvement in the time, path length or directness of their routes to the target (Fig. 2, black traces).

To verify that flies are using the spatially distinct features of the visual panorama to direct navigation, we also tested flies using an uncoupled condition whereby the cool tile was still randomly relocated for each trial but the display remained stationary throughout. With this training regime, the visual panorama provides no consistent location cues, but idiothetic and weaker spatial cues such as the distance and local orientation of the arena wall are still available to the flies. Our results (Fig. 2, grey traces) demonstrate that flies trained with the uncoupled visual panorama show little improvement in the time taken to find the cool tile and no improvement in the directness of their approaches. Thus, spatially relevant visual cues are required for flies to learn the location of the target.

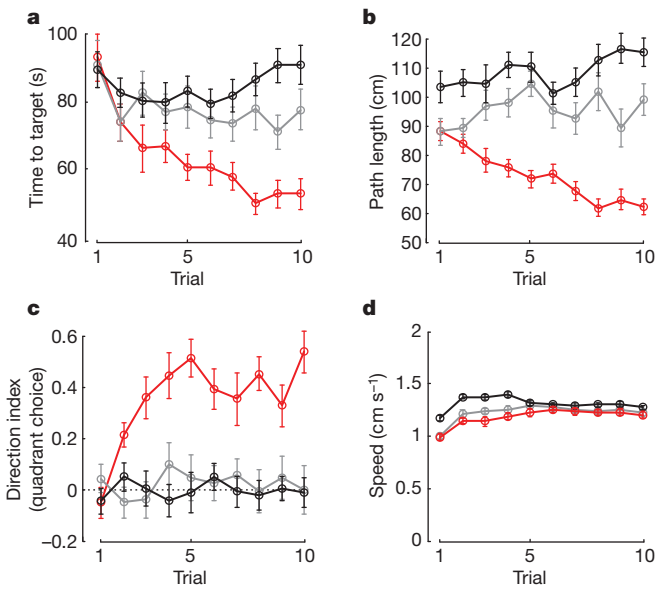

Figure 2 | Flies use visual cues to improve in place learning tasks. Flies were trained with a coupled visual panorama (red; $n = 33$ experiments, 495 flies), an uncoupled visual panorama (grey; $n = 21$ experiments, 315 flies) or in the dark (black; $n = 23$ experiments, 345 flies). **a**, When trained in the coupled condition (red) flies reduce the time taken to find the cool tile by nearly one-half, whereas flies trained with an uncoupled panorama (grey) or in the dark (black) show little or no reduction in the time taken to locate the target. **b**–**d**, The improvement seen for the coupled visual panorama is due to flies taking shorter (**b**), more direct (**c**) paths to the target rather than simply increasing walking speed (**d**). See Methods for details of calculations. Values shown are mean ± s.e.m.

As a further test of visual place memory, flies were challenged immediately after training with a probe trial (trial 11) in which the visual landscape is relocated as usual but no cool tile is provided (to determine whether the flies will go to the non-existent safe spot). We proposed that if the flies learned to locate the cool tile by using the peripheral visual landmarks, then they should bias their searches to the area of the arena where the visual landscape indicates the cool tile should be, even when the target is absent. Indeed, flies preferentially search in the arena quadrant where they have been trained to locate the now 'imaginary' cool tile (Fig. 3, Supplementary Figs 2 and 3a, and Supplementary Movie 2). In contrast, if flies were trained in the dark or with an uncoupled visual landscape, conditions that contain no specific information about the location of the cool tile, the flies instead searched the arena uniformly during the probe trial (Fig. 3c, Supplementary Figs 2 and 3a, and Supplementary Movie 3). Together, these results demonstrate that fruit flies can learn spatial locations on the basis of distal visual cues and use this memory to guide navigation. By varying the time between the end of a single round of training (ten trials) and testing during a probe trial, we could also show that flies retain these visual place memories for at least 2 h (Fig. 3d).

We next considered where spatial memories are processed (or stored) in the *Drosophila* brain. We reasoned that specific regions of the fly brain would function as the neuroanatomical substrate for

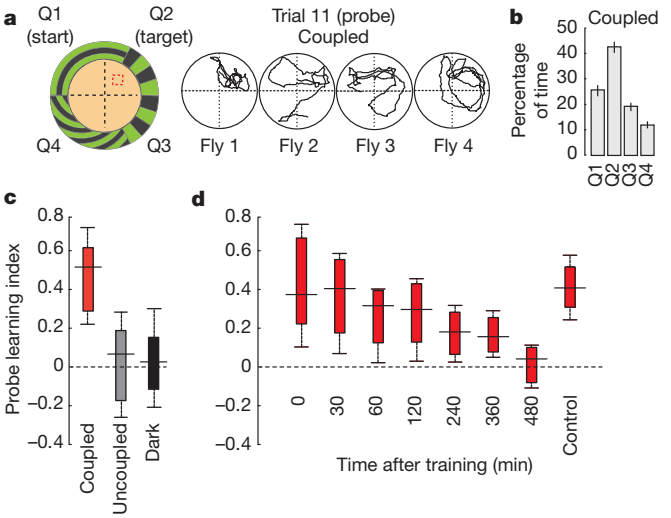

Figure 3 | Trained flies show a persistent search bias in the absence of the cool tile and retain this memory for several hours. Flies are tested in a probe trial (trail 11) in which the visual display is relocated but no cool tile is present. **a**, Trajectories from four representative flies, each plotted for 60 s after leaving its starting quadrant. Flies start in Q1; the dashed square denotes the 'expected' location of the cool tile, in Q2. **b**, Flies preferentially search in the quadrant where they have been trained to find the cool spot (Q2), even when the cool spot is absent. Values shown are mean ± s.e.m.; $n = 33$ experiments, 495 flies. **c**, Probe learning index is significantly greater than zero (indicating learning) when flies are trained with a coupled visual panorama (red; $P < 0.0001$, $n = 33$), but not when they are trained with an uncoupled visual panorama (grey; $P = 0.28$, $n = 21$) or in the dark (black; $P = 0.39$, $n = 23$). **d**, To test place memory retention, flies were tested in a probe trial at several time intervals following training ($n \geq 5$). Flies retain visual place memories for at least 2 h after training. Because flies were left in the arena between training and testing (for up to 8 h), 'control' refers to siblings placed in the arena for an equivalent amount of time (8 h) before training followed by immediate testing. Box plots indicate the median value (solid black line), 25th and 75th percentiles (box), and the data range (whiskers). For details of calculations and additional statistics, see Methods.

visual place learning, and we therefore set out to engineer and test animals in which different brain areas were selectively inactivated using the GAL4/UAS expression system. In essence, we conditionally silenced small subsets of neurons in adult flies by targeting expression of the inward-rectifying potassium channel Kir2.1[10]; to limit potential side-effects of Kir2.1 expression during development, we used a temperature-sensitive *GAL80^{ts}* that blocks Kir2.1 expression when flies are reared at 18 °C but allows expression when the temperature is raised to 30 °C before testing[11]. *GAL4* driver lines were selected for expression in two areas: the mushroom bodies (Fig. 4a–c) and the central complex (Fig. 4d–f). The mushroom bodies have been the subject of extensive studies of learning and memory in *Drosophila*[12], and have been shown to be essential for associative olfactory conditioning[13] but not for some other forms of learning, such as tactile, motor and non-visually guided place learning[14–16]. The central complex is thought to be a site of orientation behaviour, multisensory integration and other 'high-order' processes[17,18]. In some social insects, the mushroom bodies have been implicated in visual place learning[19,20], and in the cockroach bilateral surgical lesions to these structures abolish spatial learning[1]. However, we see no evidence for involvement of the mushroom bodies in our assay. In fact, silencing mushroom body intrinsic neurons using the *GAL4* drivers R9A11, R10B08, R67B04 (Fig. 4a–c, g and Supplementary Fig. 3b) and OK107, or even by chemically ablating the mushroom bodies (using hydroxyurea[13]; Supplementary Fig. 4), had no significant effect on the performance of flies in visual place learning. The differing requirement for the mushroom bodies between *Drosophila* and other species may be explained by the observations that mushroom body inputs in *Drosophila* are predominantly olfactory[1,21]. In sharp contrast, silencing subsets of neurons with projections to the central complex ellipsoid body (lines R15B07 and R28D01) dramatically impaired visual place learning (Fig. 4d–h and Supplementary Fig. 3b). Notably, silencing a different subset of ring neurons with line R38H02 leaves visual place learning intact (Fig. 4f–h). Thus, specific circuits within the ellipsoid body (but not the entire structure) are necessary for visual place learning.

To confirm that silencing the ellipsoid body neurons in lines R15B07 and R28D01 produces a specific impairment in visual place memory, we tested these flies in a series of behavioural paradigms and showed they display normal locomotor, optomotor, thermosensory and visual pattern discrimination behaviours (Fig. 4i–l and Supplementary Fig. 5). In addition, we reasoned that if these flies have a general defect in memory (or in processing thermally driven learned behaviours), then they should show impairment in multiple types of learning (or in using thermal signals to drive learning and memory). Thus, we developed a novel olfactory conditioning paradigm using temperature (rather than electric shock[22]) as the unconditioned stimulus (Fig. 4m and Supplementary Fig. 6). As expected, silencing the mushroom bodies leads to a total loss of odour learning (Fig. 4m). In contrast, silencing subsets of neurons in the ellipsoid body has no effect on olfactory learning yet ablates visual place learning. Taken together, these results demonstrate that subsets of cells in the ellipsoid body are specifically required for visual place learning and substantiate the presence of distinct neuroanatomical substrates for visually guided spatial (place) versus non-spatial (olfactory) learning in *Drosophila*.

Mammals probably use place, grid and head direction cells to solve and perform navigational tasks[8]. The tight correlation between place

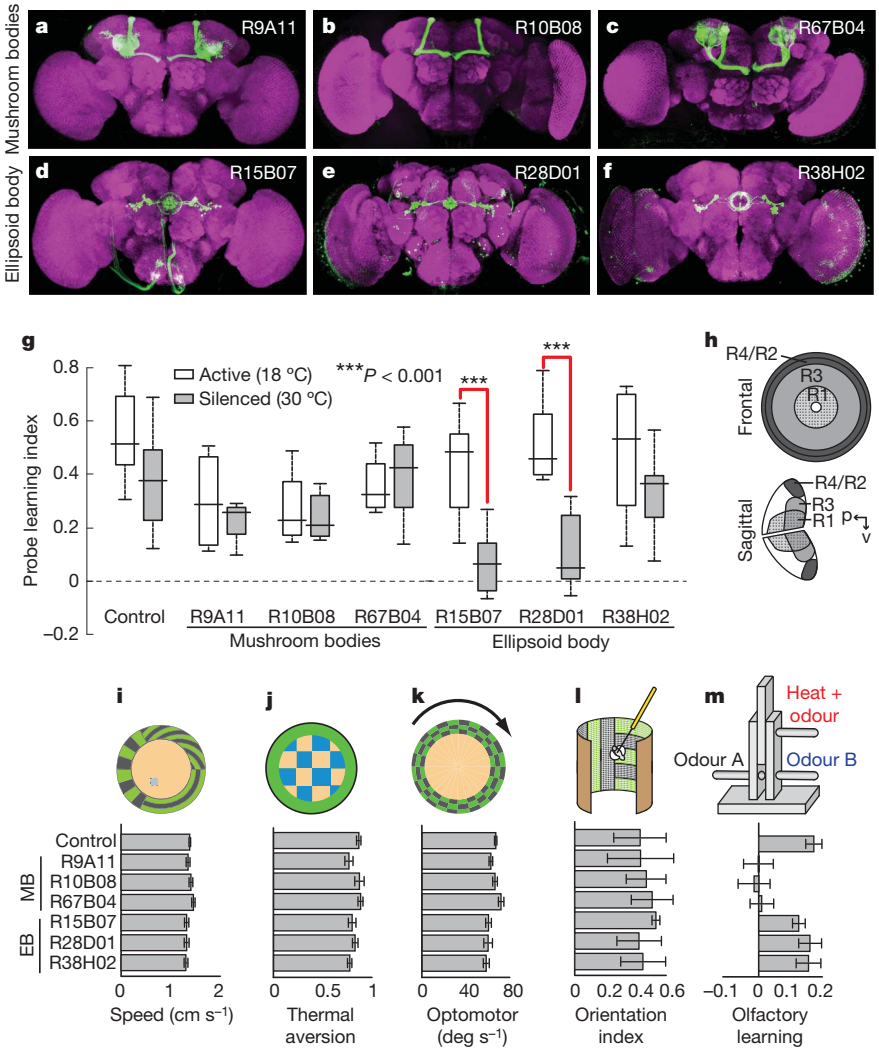

Figure 4 | Subsets of ellipsoid body ring neurons are required for place learning. **a**–**f**, *GAL4* driver lines targeting subsets of cells in the mushroom bodies (**a**–**c**) or the ellipsoid body (**d**–**f**); shown are the expression patterns for each driver using a green fluorescent protein reporter. **g**, White boxes indicate spatial learning before Kir2.1 induction; grey boxes indicate performance following Kir2.1 expression. Silencing ellipsoid body neurons projecting to R1 and R4 (R15B07) or to R1 alone (R28D01) severely impairs place learning ($P < 0.001$, red lines), whereas silencing mushroom body neurons or a separate subset of ellipsoid body neurons projecting to R4 alone (R38H02) leaves place learning intact. Box plots are as described in Fig. 3; $n \geq 8$ experiments. **h**, Schematic representation of ellipsoid body ring neuron anatomy. p, posterior; v, ventral. **i**–**m**, Flies with impaired place learning (expressing Kir2.1) show normal walking speed (**i**), heat aversion (**j**), optomotor response (**k**), visual pattern discrimination during tethered flight (**l**) and olfactory learning (**m**). In **i**–**k** ($n \geq 8$ experiments), values shown are mean ± s.e.m. In **l** ($n \geq 6$ flies) and **m** ($n = 8$ experiments), values shown are mean ± s.d. See Methods for details of calculations and additional statistical analysis. MB, mushroom body; EB, ellipsoid body.

cell activity and an animal's position in space has established the hippocampus as the substrate for a cognitive map. This map is probably informed by head direction cells (indicating an animal's orientation) and grid cells that tile the surrounding environment and could support path integration. Although it is not known whether there are direct correlates to these cells in flies, invertebrates are capable of solving similarly challenging navigational feats and do so using significantly smaller brains. Indeed, flies are able to use idiothetic cues, and path integration, to aid navigation[15,17,23,24]. Our studies now demonstrate that *Drosophila* can learn and recall spatial locations in a complex visual arena and do so with remarkable efficacy.

We also show that subsets of neurons in the fly brain (ring neurons of the ellipsoid body) are critical for visual place learning, probably by implementing, storing, or reading spatial information. Strikingly, flies in which we silenced ellipsoid body neurons have a basic 'circling' search routine (Supplementary Fig. 2d) that is reminiscent of the behaviour displayed by rats with hippocampal lesions[25]. Imaging of neuronal activity in the fly brain while the animal is executing a navigation task should help further define the role of the central complex, and ellipsoid body neurons in particular, in spatial memory (for example in a head-fixed preparation with a virtual-reality arena[26,27]). Ultimately, elucidating the cellular basis for place learning in *Drosophila* will help uncover fundamental principles in the organization and implementation of spatial memories in general.

Note added in proof: While our paper was under review, another study reported the use of a heat maze to study spatial search strategies in *Drosophila*[28].

METHODS SUMMARY

To control the thermal landscape, we developed an array of 64 1-inch-square, individually addressable thermoelectric modules arranged in an 8×8 grid (Fig. 1b). This array forms the floor of our test arena and is covered with black masking tape. To confine flies to this surface, a 3-mm-high, 8-inch-diameter, heated aluminium ring was placed around the outer perimeter of the arena and covered with a glass disk with a slippery surface. Visual cues were provided by a light-emitting-diode display positioned around the outer perimeter of the arena[29] (Fig. 1a). The experimental protocol included ten training trials (5 min each) followed by a probe trial (trial 11) in which the visual display was relocated in the absence of a cool spot. The navigational behaviour of the flies during a session was tracked off-line using CTRAX[30]. At the end of the experiment, flies were tested in the same arena for thermal preference and optomotor behaviour to confirm normal thermal and visual responses.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions All authors designed the study and wrote the manuscript. T.A.O. carried out the experiments and data analysis.

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METHODS

Thermal–visual place learning arena. To control the thermal landscape, we developed an array of 64 1-inch-square, individually addressable thermoelectric modules arranged in an 8×8 grid (Oven Industries) (Fig. 1b). This array forms the floor of our test arena and is covered with black masking tape to create a uniform, featureless surface that can be replaced between experiments. Importantly, no thermal gradients exist that could guide flies to the cool spot from a distance (Fig. 1b and Supplementary Fig. 7), as confirmed by thermal imaging (Optotherm) and thermocouple measurements. Additionally, the absence of place learning in the dark and in the uncoupled condition (evidenced by near-zero direction and probe learning indices; Figs 2c and 3c) confirms that there are no significant non-visual or idiothetic cues in the arena that guide flies to the cool spot.

To confine flies to this surface, a 3-mm-high, 8-inch-diameter aluminium ring was placed around the outer perimeter of the arena and covered with a glass disk coated with a slippery silicon film (Sigmacote). To keep flies from walking on the walls, the aluminium ring was heated to $>50\,^{\circ}\mathrm{C}$ using insulated resistance wire (Pelican Wire, 29 AWG Nichrome 60 w/Kapton). Peripheral visual cues were provided using an electronically controlled light-emitting-diode (LED) display positioned around the outer perimeter of the arena[29] (Fig. 1a). In visual place learning trials, the LED panels were set to display a visual landscape composed of evenly spaced vertical, horizontal and diagonal bars. When viewed from the arena's centre, the width of each bar covered 15° of the LED display. When viewed from a distance of 8.8 inches, the maximum distance possible in our arena, each visual element subtends $\sim 8^{\circ}$ of the fly's visual field and should be easily resolvable by the fly. The entire arena is illuminated with infrared light (Smart Vision Lights) and fly activity was recorded with a Basler 622f CMOS camera fitted with an infrared passing filter.

Visual place learning protocol and analysis. The experimental protocol included ten training trials (5 min each) followed by a probe trial (trial 11) in which the visual display was relocated in the absence of a cool spot. At the end of the experiment, flies were tested in a temperature preference trial[31,32] and an optomotor trial[33] to measure normal thermal and visual responses. Flies were tracked offline using CTRAX fly-tracking software[30]. Fly centroid data were imported into MATLAB (Mathworks) and processed using custom scripts. In Fig. 2, the time to target, path length and speed are calculated, per fly, for the time window from the start of the trial until each fly reaches the target tile. Direction index (quadrant choice) is calculated as the number of flies that first enter the quadrant containing the cool tile (number correct) minus the number of flies that first pass into the opposite quadrant (number incorrect), divided by the total number of flies. To test whether flies show a bias for certain quadrants or rotation directions during training in the visual place learning arena, we tested for the dependence of the time to target on the target quadrant and on the rotation direction (clockwise or anticlockwise). This was accomplished by calculating the difference between the time to target for each trial (from each experiment) and the mean time to target for each trial across all experiments. The differences from the mean for training to quadrants 1, 2, 3 and or 4 are 1.7 ± 2.3, -0.5 ± 2.7, 0.0 ± 2.2 and -1.2 ± 2.2 s, respectively. The differences from the mean for clockwise and anticlockwise rotations are 0.6 ± 1.5 and -0.6 ± 1.8 s, respectively. Error is reported as s.e.m. For both tests, there are no statistically significant comparisons using one-way analysis of variance (ANOVA) at $P < 0.05$.

In Fig. 3b, the percentage of time spent in each of the quadrants was tested for statistical significance using one-way ANOVA with a Bonferroni correction for multiple comparisons. Flies spend significantly more time searching in Q2 than in Q1, Q3 or Q4 ($P < 0.01$). The probe learning index in Figs 3 and 4 is calculated from probe trial trajectories as the amount of time during the first 60 s after leaving the starting quadrant that flies spent searching in Q2 (the quadrant where they have been trained to locate the cool tile) minus the amount of time spent searching in Q4 (a quadrant that is the same distance from the starting quadrant, but in the wrong direction), divided by the total time spent in both quadrants. The P values reported in the legend of Fig. 3c were calculated using a one-tailed t-test. The probe learning index scores in Fig. 3d were tested for statistical significance ($P < 0.05$) using one-way ANOVA with a Bonferroni correction for multiple comparisons and pairwise comparisons to the uncoupled condition in Fig. 3c. The probe learning scores are significantly greater than uncoupled for up to 120 min after training. We note that although the probe learning index is not significantly different from the uncoupled control at 4 and 6 h, it is significantly greater than zero (one-tailed t-test, $P < 0.05$). Because flies were left in the arena between training and testing (for up to 8 h), 'control' refers to siblings placed in the arena for an equivalent amount of time (that is, 8 h) before training followed by immediate testing. The P values reported in the legend of Fig. 4 were calculated using a one-tailed t-test comparing place learning scores before (white box) and after (grey box) Kir2.1 induction. The probe learning index scores reported in Fig. 3c and 4g were also tested for statistical significance using one-way ANOVA with a Bonferroni correction for multiple comparisons. In Fig. 3c, flies trained in the coupled condition show significantly higher probe learning scores ($P < 0.01$) when compared with flies trained in the uncoupled condition or in the dark. There is no significant difference (at the $P < 0.05$ level) between uncoupled and dark. In Fig. 4g, R15B07 and R28D01 flies shifted to 30 °C are significant at $P < 0.01$ when compared with control flies. No other comparisons with control flies are significant at $P < 0.05$. In all box and whisker plots, the whiskers cover the range of the data, excluding outliers. Outliers are defined as data points greater than the 75th percentile of all data points plus 1.5 times the interquartile range ($q_3 + 1.5(q_3 - q_1)$) or data points less than the 25th percentile of all data minus 1.5 times the interquartile range ($q_1 - 1.5(q_3 - q_1)$). The majority of data sets presented as box plots contain no outlying data.

Following the probe trial, flies were tested for thermal preference by setting alternating tiles on the array of thermoelectric modules to either 25 or 36 °C. Flies were allowed to distribute for 2 min before the cool and warm tiles were switched. The flies were then allowed another 2 min to redistribute, and the thermal aversion index was calculated as the amount of time flies spent at 25 °C minus the amount of time spent at 36 °C, divided by the total time. Finally, flies were tested for normal optomotor responses by rotating a chequerboard pattern on the visual panorama clockwise and then anticlockwise at $90^{\circ}\,\mathrm{s}^{-1}$ for 45 s. Optomotor responses are reported as the mean rotational speed (in the direction of the stimulus) of the flies over the course of these trials. No significant differences are observed in thermal aversion or optomotor response at $P < 0.05$ using one-way ANOVA with a Bonferroni correction for multiple comparisons.

Non-visual place learning. Work by a number of groups[15,17,23,24,34–36] has shown that flies can use idiothetic cues and path integration to navigate (in several forms of non-visual place memory task). To address whether idiothetic (that is, non-visual) cues are sufficient to guide navigation in the thermal–visual arena, we tested flies using a set of modified training protocols that included (a) keeping the cool tile stationary, (b) rotating the cool tile in a constant direction and (c) randomly rotating the cool tile between trials, all in the dark. Next we tested flies with (d) a stationary visual panorama and a randomly relocated cool tile, (e) a randomly rotating visual panorama and a stationary cool tile, and (f) random, independent relocations of the visual panorama and cool tile (Supplementary Fig. 8). We see no evidence of place learning when flies are trained and tested with any of these modified protocols. We note that flies may use idiothetic information while navigating; however, this experience is not sufficient for the formation of a place memory. To disperse flies from the stationary cool tile between trials, the entire array was heated to 36 °C for 60 s before the start of each trial. When trained with the standard coupled visual panorama (Figs 1 and 2), this manipulation does not impair visual place learning (data not shown).

Olfactory conditioning. Olfactory conditioning experiments were based on experiments using an elevated T-maze as described in ref. 22. The conditioning protocol was modified to use temperature rather than electric shock as the unconditioned stimulus (Fig. 4m and Supplementary Fig. 6). During conditioning, the training tube was heated to 36 °C concurrent with delivery of the first odour by passing a 5-V, 0.43-A current through a custom-built mesh of insulated resistance wire (Pelican Wire, 29 AWG Nichrome 60 w/Kapton) inserted into the training tube. Odours were delivered by bubbling an air stream through a vial containing odorant diluted in paraffin oil. Odours used were 5% 4-methylcyclohexanol (MCH; flow rate, 128 ml min^{-1}) and 5% 3-octanol (OCT; flow rate, 60 ml min^{-1}). Flow rate through training and testing tubes was normalized to 800 ml min^{-1} by combining the odorant stream with a humidified clean air stream. About 200 flies were tested in each experiment, one half conditioned to MCH and the other conditioned to OCT. Learning indices were calculated as the average learning index of the two groups. All mushroom body lines (R9A11, R10B08 and R67B04) are significantly impaired in olfactory learning when compared with control flies ($P < 0.05$ using one-way ANOVA with a Bonferroni correction for multiple comparisons). No ellipsoid body lines are significantly different from control.

Tethered flight experiments. Closed-loop tethered flight experiments were performed as previously described using a cylindrical LED display and an optical wing-beat analyser to measure fly responses[29]. To test whether flies were capable of discriminating the visual features of the panoramic pattern in the visual place learning arena, we examined the orientation preference of flying flies for a flight arena pattern that was composed of four quadrants that display 15°-wide bar gratings, in either a vertical (quadrants 1 and 3) or horizontal (quadrants 2 and 4) direction. Each fly was allowed to selectively orient in a behavioural closed loop with this pattern for five trials of 50 s each, as part of an experimental series consisting of other closed- and open-loop trials, for which no further data are shown. Flies showed a clear preference for the vertical bars, so we quantified the behaviour with an orientation index that was calculated as the amount of time flies

oriented towards vertical bars minus the amount of time they oriented towards horizontal bars, divided by the total time. No significant differences are observed in the orientation index at $P < 0.05$ using one-way ANOVA with a Bonferroni correction for multiple comparisons.

Experimental animals. All flies used were female and, unless otherwise noted, are DL wild type. This strain is a laboratory culture produced by interbreeding dozens of wild caught isofemale lines, was established in 1995 and is maintained by Michael Dickinson's laboratory[37]. Flies were reared on standard media at 25 °C on a 16-h/8-h light/dark cycle. Visual place learning experiments were performed with 4-d-old adult flies during hours 11–15 of the flies' subjective day (where hour 0 corresponds to the transition from dark to light) in a room kept at 25 °C and 40% relative humidity. For neural silencing experiments, *w^+;tubP-GAL80^{ts};UAS-Kir2.1* flies (backcrossed ten generations into DL wild-type genetic background to control for the effects of genetic background[38] and known behavioural deficits with flies homozygous for *w^{1118}* (ref. 36)) were crossed to *GAL4* driver lines and reared at 18 °C. Two-day-old adult females were temperature-shifted to 30 °C for 40 h and then returned to 18 °C for 2 h before testing. *GAL4* driver lines were constructed as described in ref. 39 and were provided by Gerry Rubin. Control flies are *w^{1118};attP2* (the same genetic background as the *GAL4* lines) crossed to *w^+;tubP-GAL80^{ts};UAS-Kir2.1*. To ensure that backcrossing into the DL genetic background does not create conditions for PM hybrid dysgenesis (that is, mobilization of p-elements), our effectors/reporters (all marked with *mini-white*) are kept over balancer chromosomes and we regularly monitor for the appearance of the reporter in the wrong chromosome (an indication of transposition). Over the three years that we have been crossing and monitoring these stocks, we see no evidence of transposition. In Supplementary Fig. 5, line 78y was included to highlight locomotor abnormalities in a line with documented motor impairment[40].

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