

LETTERS

Cryptochrome mediates light-dependent magnetosensitivity in *Drosophila*

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Although many animals use the Earth's magnetic field for orientation and navigation^{1,2}, the precise biophysical mechanisms underlying magnetic sensing have been elusive. One theoretical model proposes that geomagnetic fields are perceived by chemical reactions involving specialized photoreceptors³. However, the specific photoreceptor involved in such magnetoreception has not been demonstrated conclusively in any animal. Here we show that the ultraviolet-A/blue-light photoreceptor cryptochrome (Cry) is necessary for light-dependent magnetosensitive responses in *Drosophila melanogaster*. In a binary-choice behavioural assay for magnetosensitivity, wild-type flies show significant naive and trained responses to a magnetic field under full-spectrum light (~300–700 nm) but do not respond to the field when wavelengths in the Cry-sensitive, ultraviolet-A/blue-light part of the spectrum (<420 nm) are blocked. Notably, Cry-deficient *cry⁰* and *cry^b* flies do not show either naive or trained responses to a magnetic field under full-spectrum light. Moreover, Cry-dependent magnetosensitivity does not require a functioning circadian clock. Our work provides, to our knowledge, the first genetic evidence for a Cry-based magnetosensitive system in any animal.

The ability of an animal to detect geomagnetic fields has substantial biological relevance as it is used by many invertebrate and vertebrate species for orientation and navigation purposes, including homing, building activity and long-distance migration^{2,4}. Three general modes of magnetoreception have been proposed⁵. One mode is electromagnetic induction by the Earth's magnetic field, which may occur in electrosensitive marine fish, although there is little evidence to support such sensing. The two other modes, for which experimental evidence does exist, are a magnetite-based process^{6–8} and chemical-based reactions^{9,10} that are modulated by magnetic fields. One chemical model of magnetoreception proposes that magnetic information is transmitted to the nervous system through the light-induced product of magnetically sensitive radical-pair reactions in specialized photoreceptors³.

Cry proteins are flavoproteins that have been postulated to generate magnetosensitive radical pairs that could provide a photoinduced electron transfer reaction for the detection of magnetic fields³. Cry proteins are best known for their roles in the regulation of circadian clocks^{11,12} and can be categorized into two groups on the basis of current phylogenetic and functional relationships^{13,14}. *Drosophila*-like Cry proteins are sensitive to light in the ultraviolet-A/blue range¹⁵ and function primarily as photoreceptors that synchronize (entrain) circadian clocks. Vertebrate-like Cry proteins, which have also been found in every non-drosophilid insect so far examined¹⁴, do not seem to be directly light-sensitive. Instead, vertebrate-like Cry proteins are potent repressors of the Clock and Bmal1 (known as Cycle in insects) transcription factors which, as heterodimers, drive the intracellular transcriptional feedback loop of the circadian clock mechanism in all animals studied.

Although there is good behavioural evidence for the involvement of short-wavelength photoreceptors in the detection of a geomagnetic field^{5,16–18}, an essential link between Cry and magnetoreception has not been established in any animal. *Drosophila* are ideally suited to investigate a role for Cry as a magnetoreceptor, because they only have the light-sensitive Cry¹⁴ in which the action spectrum peaks in the ultraviolet-A range (350–400 nm) with a plateau in the near blue range (430–450 nm)^{19,20}. Notably, flies that lack Cry (*cry⁰*)²¹ or harbour the chemically induced missense *cry^b* mutation^{22,23} can be used to evaluate the role of Cry in magnetosensitive responses.

We initiated our studies by developing a behavioural assay for magnetosensitivity in *Drosophila* (Fig. 1a). In this illuminated apparatus, flies experience a magnetic field generated by an electric coil system and display their magnetosensitivity in a binary-choice T-maze. The two-coil system is ideal for behavioural studies of magnetosensitivity, because it produces a magnetic field on one side of the T-maze, while producing no field on the opposite side. This design eliminates non-magnetic differences such as heat generated by the electric coils between sides during test sessions²⁴. Flies were tested either for their response to the magnetic field in the naive state (naive group) or after a training session pairing the field with sucrose reward (trained group).

Wild-type Canton-S, white-eyed *w¹¹¹⁸*Canton-S, Oregon-R and Berlin-K strains all developed a learned preference for a magnetic field (Fig. 1b). The trained groups in the two Canton-S lines showed the greatest response to the field ($P = 0.002$, one-way analysis of variance (ANOVA)) and were the only ones to show a naive avoidance of the field ($P < 0.0001$, one-sample *t*-test). Thus, *Drosophila* consistently show magnetosensitivity that varies in magnitude in a strain-dependent manner. The similarity of behavioural responses between red-eyed, wild-type Canton-S flies and white-eyed *w¹¹¹⁸*Canton-S flies shows that eye colour does not substantially alter behavioural responses to the magnetic field.

Because wild-type Canton-S flies showed the most robust trained and naive responses of the strains tested, we used them to determine whether the magnetic responses we observed were light-dependent. We assayed naive and trained Canton-S flies under different long-wavelength pass filters that transmitted wavelengths of light at >500 nm, >420 nm or >400 nm (Fig. 2a). In contrast to flies assayed under full-spectrum light (Fig. 1b and Fig. 2a), flies did not show either naive or trained responses to the field when wavelengths <420 nm were blocked (Fig. 2b). Because the filter that blocked light <420 nm also caused a 13% decrease in total irradiance (Fig. 2c, red line), we examined whether the filter-induced lack of behavioural responses to the magnetic field was secondary to the decrement in irradiance. When Canton-S flies were studied under full-spectrum light, with a total irradiance level lower than that imposed by the filter (Fig. 2c, blue line), the flies still showed significant naive ($P = 0.0005$, one-sample *t*-test) and trained responses to

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the magnetic field (Fig. 2d). Thus, the filter-induced loss of behavioural responses to the magnetic field is due to the loss of short-wavelength light.

Behavioural responses to the magnet were partially restored when 400–420 nm light was included (Fig. 2b), which is consistent with the action spectrum of *Drosophila* Cry tailing into the near blue¹⁹, and, as expected, the trained response was weaker than that under full-spectrum light (full spectrum versus >400 nm, $P < 0.001$, Student's t -test). This wavelength-dependent effect of the magnetic field on behaviour suggests that *Drosophila* has a photoreceptor-based magnetosensitive system. Moreover, because the response to the magnetic field requires ultraviolet-A/blue light (<420 nm; Fig. 2e), these

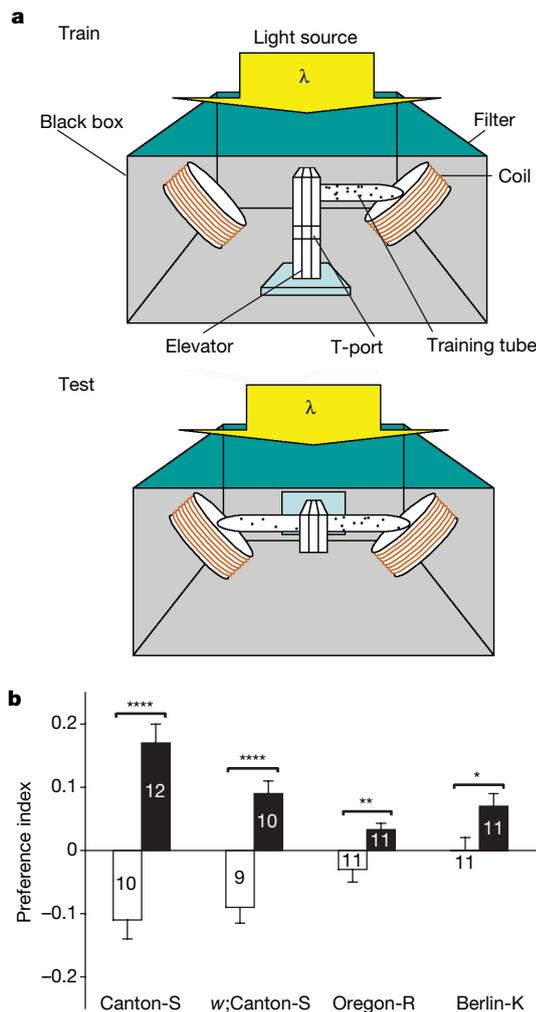


Figure 1 | Behavioural apparatus for magnetosensitivity and behavioural responses in different *Drosophila* strains. **a**, Behavioural apparatus for magnetosensitivity. The top diagram (Train) shows the frontal view of the choice chamber apparatus positioned for training. The chamber apparatus consisted of a training tube, an elevator to transfer flies, and a duel-choice point (T-port). For training, the apparatus, with training tube only, was placed upright in an illuminated black box containing a two-coil system. A population of flies (dots) was loaded into the training tube with or without sucrose reinforcement and a magnetic field. The bottom diagram (Test) shows the frontal view of the choice chamber apparatus positioned for testing. For testing, the apparatus, with tubes attached to the T-port (T-maze), was rotated to the horizontal and flies were transferred from the elevator section to the T-port. Wavelength dependence was examined using long-wavelength pass filters. **b**, *Drosophila* strains vary in their behavioural response to a magnetic field under full-spectrum light (Fig. 2a). Bars show the preference index of the naive (white) or trained (black) groups. Numbers represent the groups tested and values are mean \pm s.e.m. *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$.

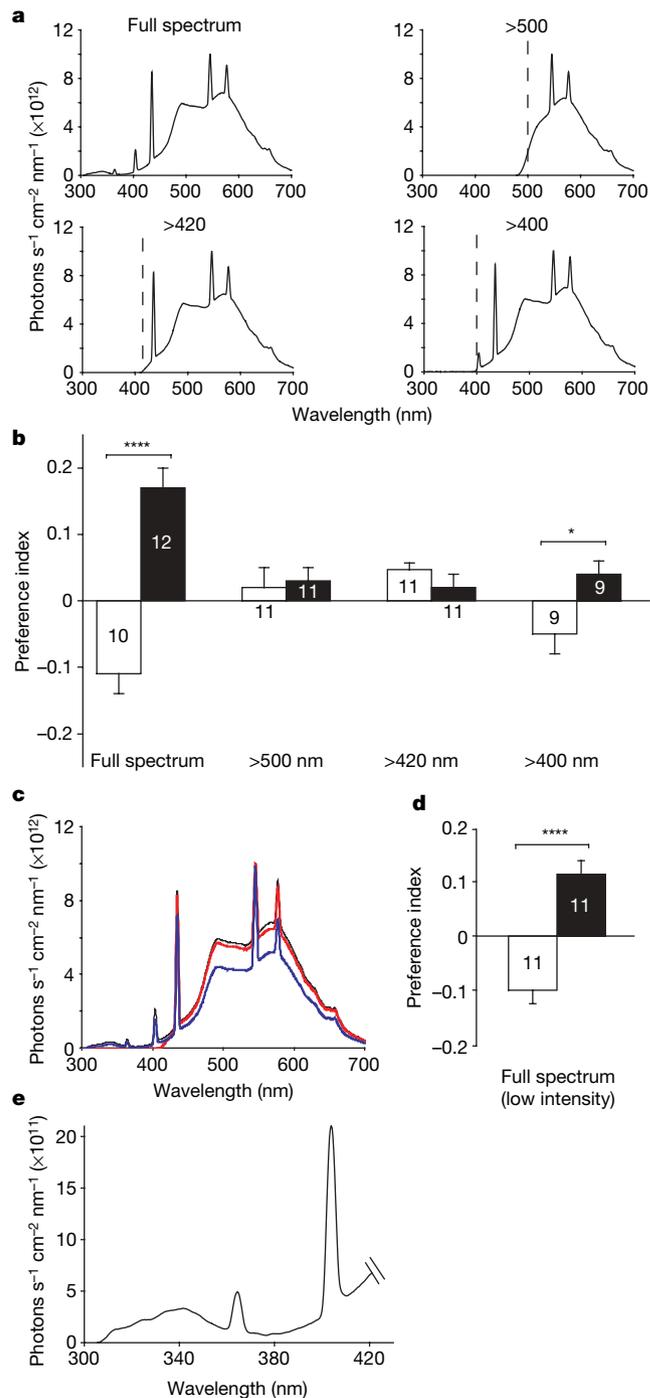


Figure 2 | Short-wavelength light is required for magnetosensitivity in Canton-S flies. **a**, Irradiance curves for different light conditions. Light measurements were taken inside the training and test tube. Dashed lines denote cutoff points of the blocking filters. **b**, Wavelength-dependence of the magnetic response. Bars show the preference index of the naive (white) or trained (black) groups. Full-spectrum data are from Fig. 1b. Numbers represent the groups tested. *, $P < 0.05$; ****, $P < 0.0001$. **c**, Irradiance curves depicting full-spectrum light (black line), light >420 nm (red line) and full-spectrum light with reduced total irradiance (full spectrum, low intensity; blue line). **d**, Canton-S flies still elicited significant responses to the magnetic field under full-spectrum, low intensity light. ****, $P < 0.0001$. **e**, Irradiance values from 300–420 nm. Data are expanded scale from full-spectrum pattern in **a**. The irradiance values in ultraviolet-A/blue light in our studies (300–420 nm) are in line with those reported for *Drosophila* Cry function using other biological responses^{19,20}, that is, a range of 10^{11} to 10^{12} photons $s^{-1} cm^{-2} nm^{-1}$. Values from **b** and **d** are mean \pm s.e.m.

data are consistent with the hypothesis that Cry can function as a magnetoreceptor in *Drosophila*.

We next used Cry-deficient *cry⁰* mutant flies to examine directly whether Cry is required for magnetosensitive behaviour. We tested two of the newly generated *cry⁰* fly lines, because, in *cry⁰* flies, the entire *cry* coding sequence has been replaced with mini-*white⁺* by homologous recombination, ensuring that, unlike in the more commonly used point-mutant *cry^b* flies, there is no possibility of residual Cry activity²¹. In addition, the three *cry⁰* fly lines (from *cry⁰¹* to *cry⁰³*) were backcrossed independently into a *w¹¹¹⁸* background²¹. Thus, we were able to use the appropriate *w¹¹¹⁸* control flies to test the contribution of the *cry* gene in magnetosensitive behaviour.

Control *w¹¹¹⁸* flies showed a clear naive preference for, rather than avoidance of, the magnetic field (Fig. 3a). The difference in the direction of the naive response to the magnetic field between Canton-S flies and the *w¹¹¹⁸* line re-emphasizes the importance of controlling for genetic background in studies of magnetosensitivity in flies. Nonetheless, like Canton-S flies, the naive response of *w¹¹¹⁸* flies to the magnetic field was light dependent; the naive preference for the magnetic field was abolished in the absence of ultraviolet-A/blue light (<420 nm; Fig. 3a).

Homozygous *cry⁰²* flies lacking Cry did not show a naive response to the magnet under full-spectrum light, in contrast to the significant naive responses manifested by both *w¹¹¹⁸* and heterozygous *cry^{02/+}* flies (Fig. 3b). Training control *w¹¹¹⁸* flies to prefer the magnetic field under full-spectrum light significantly enhanced their naive preference for the field (Fig. 3c). In contrast, homozygous *cry⁰¹* flies did not show either a naive preference for the field (like *cry⁰²* flies) or an enhanced preference for the field after training (Fig. 3c). The loss of the response to the magnetic field in the Cry-deficient flies resembled the behaviour when *w¹¹¹⁸* flies were deprived of ultraviolet-A/blue light (Fig. 3a), which is consistent with Cry being the relevant light sensor. These data using two *cry* null strains strongly suggest that both naive and trained responses to the magnetic field in *Drosophila* require Cry function.

The Cry-defective *cry^b* mutant flies are also unable to respond to the magnetic field; the *cry^b* mutation renders Cry^B essentially non-functional^{22,23}. Because the genetic background of *cry^b* mutant flies is not well defined, we compared behavioural responses to the magnetic field between homozygous *cry^b* flies and heterozygous *cry^b/Canton-S* flies. Whereas homozygous *cry^b* flies did not show either naive or trained responses to the magnetic field under full-spectrum light, heterozygous *cry^b/Canton-S* flies showed significant naive ($P = 0.0004$, one-sample *t*-test) and trained responses (Fig. 3d); the trained response in the heterozygotes was less than that of wild-type Canton-S flies (Fig. 1b) and probably results from differences in genetic background.

To rule out non-*cry* mutations as the reason for the lack of magnetic responses in *cry^b* mutants, we showed that the *cry^b* mutation fails to complement the *cry⁰¹* null mutation. Transheterozygous *cry^b/cry⁰¹* flies did not show significant naive or trained responses to the magnet, whereas heterozygous *cry⁰¹/Canton-S* and *cry^b/Canton-S* flies did (naive response, $P = 0.006$, one-sample *t*-test; Fig. 3d). Taken together, these data indicate that the *cry* locus is necessary for light-dependent magnetosensitivity in *Drosophila*. Furthermore, the lack of a trained response in both *cry⁰¹* and *cry^b* mutant flies is consistent with Cry being an essential component of the magnetosensitive sensory input pathway and perhaps the magnetoreceptor itself.

Because light-activated Cry interacts with the critical circadian clock protein Timeless to reset the circadian clock mechanism²⁵, we examined whether an intact circadian system is necessary for the Cry-dependent magnetosensitive responses in wild-type Canton-S flies. Circadian arrhythmicity was induced by constant light, which disrupts circadian clock function in Cry-containing cells by causing the constant degradation of not only Cry but also Timeless and then Period²⁵. We subsequently tested behavioural responses to the

magnetic field after at least 5 days in constant light when the flies were shown to express arrhythmic locomotor behaviour (Fig. 4a), to have disrupted Period abundance rhythms (Fig. 4b), and to express constantly low levels of Cry (Fig. 4c). Notably, these arrhythmic flies continued to show significant naive ($P = 0.004$, one-sample *t*-test)

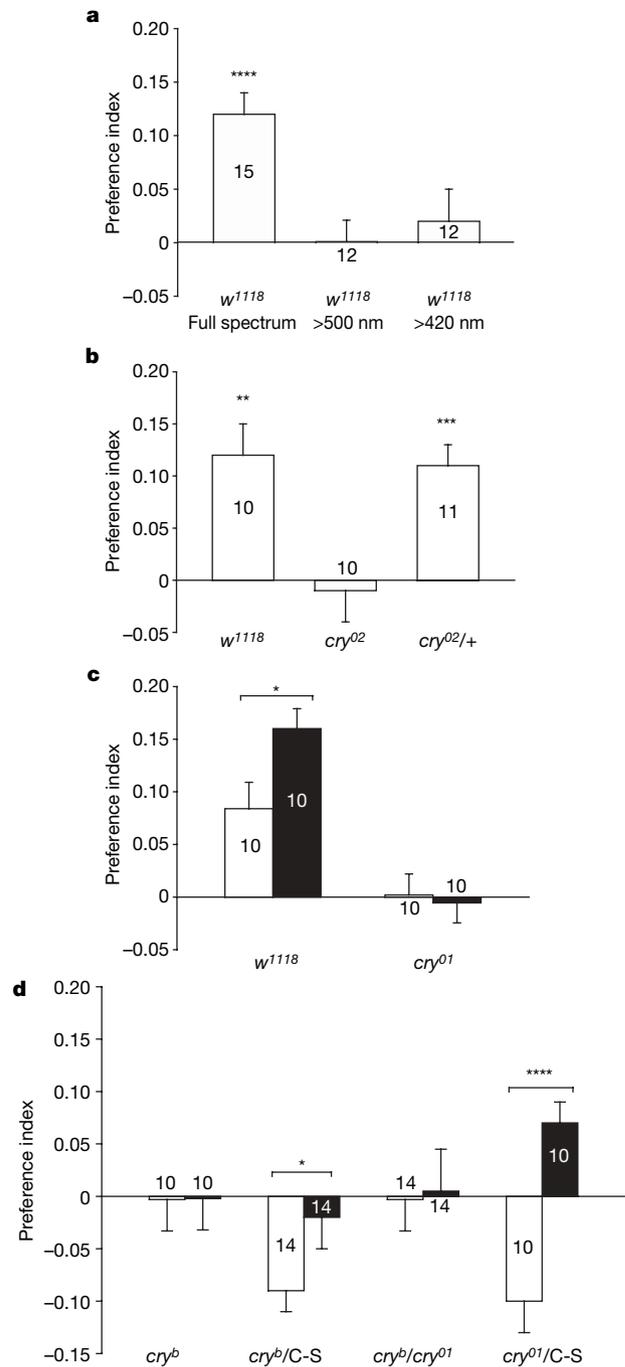


Figure 3 | *Drosophila* Cry mediates magnetosensitivity.

a, Magnetosensitivity in *w¹¹¹⁸* flies depends on ultraviolet-A/blue light. Bars show preference index values for naive responses under full-spectrum light and light >500 nm and >420 nm. Numbers represent the groups tested. ****, $P < 0.0001$. **b**, Naive response to a magnetic field is impaired in Cry-deficient *cry⁰²* flies, but not in *cry^{02/+}* flies. Bars show preference index values for naive responses. **, $P < 0.01$; ***, $P < 0.001$. **c**, Naive and trained responses to a magnetic field are impaired in Cry-deficient *cry⁰¹* flies. Bars are preference index values for naive (white) and trained (black) groups. *, $P < 0.05$. **d**, Naive and trained responses to a magnetic field are impaired in homozygous *cry^b* and transheterozygous *cry^b/cry⁰¹* flies. Bars show preference index values for naive (white) or trained (black) groups. C-S, Canton-S. *, $P < 0.05$; ****, $P < 0.0001$. Values from **a–d** are mean \pm s.e.m.

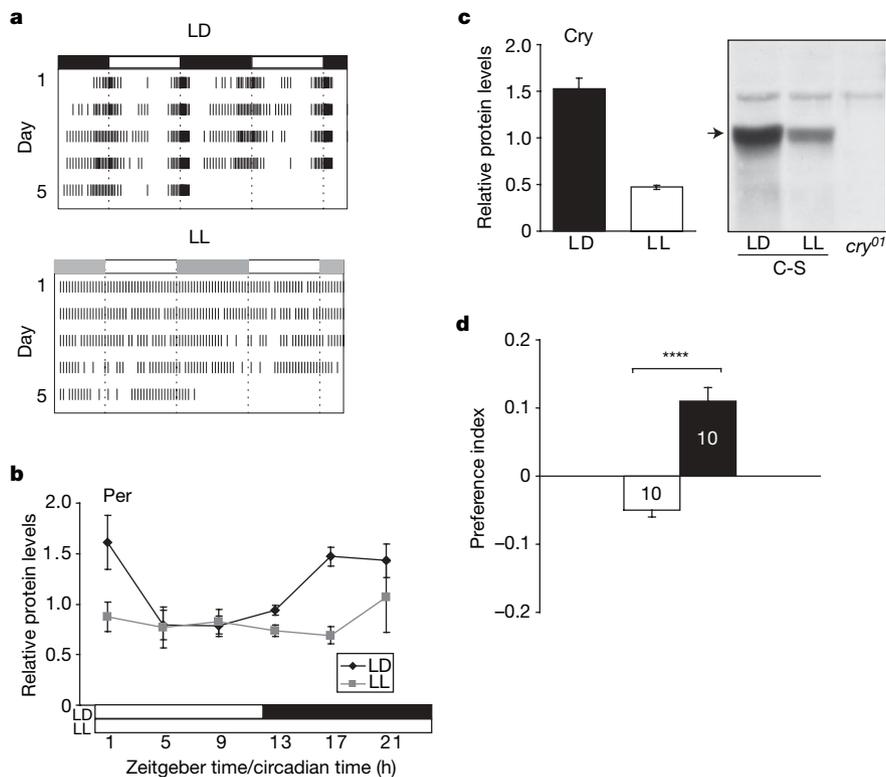


Figure 4 | Constant light disrupts circadian function but not Cry-mediated magnetosensitivity in Canton-S flies. **a**, Mean activity records in 12 h light:12 h dark lighting cycle (LD; top) or constant light (LL; bottom) in double-plotted format ($n = 62$ for each group). The lighting conditions were identical to those used for housing flies tested for responses to magnet; light irradiance, 1.5×10^{15} photons $s^{-1} cm^{-2} nm^{-1}$. For the light-dark cycle, 94% expressed circadian rhythms when released in constant darkness (period, 24.6 ± 0.03 h). All the constant-light flies were arrhythmic. **b**, Temporal profiles of Period (Per) in heads. Protein abundance was rhythmic in the

light-dark cycle ($P < 0.01$, one-way ANOVA), but not in constant light. Head extracts were analysed by western blot³⁰ and normalized against α -tubulin. Values are mean \pm s.e.m. from three sets of heads. **c**, Cry abundance is decreased in LL. Values are mean \pm s.e.m. from three sets of heads collected over 24 h in the light-dark cycle or in constant light. Right, western blot probed for Cry³⁰ showing presence (arrow) in the light-dark cycle or in constant light in Canton-S (C-S) heads, and absence in *cry⁰¹* heads. **d**, Flies in constant light elicit behavioural responses to the magnetic field. Values are mean \pm s.e.m. ****, $P < 0.0001$.

and trained responses to the magnetic field (Fig. 4d). Thus, the continuous activation of Cry by light does not disrupt its ability to sense the magnet, and an intact circadian system is not required for the magnetoreception mechanism to operate.

There are two other published reports of magnetosensitivity in adult *Drosophila*^{26,27}. One describes behavioural evidence that male wild-type Oregon-R flies show a light-dependent magnetic compass response in a radial maze whereas female flies did not respond to the magnet²⁷. Additionally, male flies responded in opposite directions when tested under either 365 nm or 500 nm light. In our studies, both male and female flies showed a magnetic response. Regardless of experimental differences, both the previous study²⁷ and ours demonstrate that fruitflies can respond to a magnetic field in a wavelength-dependent manner.

Our results extend substantially the presence of a light-dependent magnetic sense in *Drosophila* by showing the necessity of Cry. We cannot distinguish unequivocally whether fly Cry functions as the actual magnetoreceptor or whether it is an essential component downstream of the receptor. Cry is necessary for both the naive and trained responses to the magnetic field, consistent with the notion that Cry is in the input pathway of magnetic sensing. In addition, the continued behavioural responses to the magnet in constant light, in which the known Cry signalling components are being constantly degraded and the circadian clock is rendered non-functional, is also consistent with an input function. The most compelling evidence supporting a magnetoreceptor role for Cry is that the Cry-dependent behavioural responses to the magnetic field require ultra-violet-A/blue light, which matches the action spectrum of *Drosophila* Cry^{19,20}.

Our behavioural assay for magnetosensitivity does not at present have a pure directional component, and therefore it is difficult to relate our findings directly to the use of geomagnetic fields for animal orientation and navigation. Nevertheless, it is probable that the response we have identified is the prototype for the involvement of Cry in chemical-based magnetic sensing. Thus, our findings open new avenues of investigation into the cellular and molecular basis of chemical-based magnetic sensing in animals. The powerful genetics of *Drosophila* will facilitate an understanding of the precise mechanism of action of Cry in magnetosensitivity, such as the actual involvement of magnetosensitive radical pairs produced by photo-induced electron transfer reactions²⁸. Our data further show that the biological functions of *Drosophila* Cry extend beyond those in circadian clocks.

METHODS SUMMARY

Fly stocks were raised on standard cornmeal/agar medium at 25 °C and 60% relative humidity under a 12 h light:12 h dark lighting cycle. The *w¹¹¹⁸;cry⁰* flies (from *cry⁰¹* to *cry⁰³*) were a gift from J. C. Hall and are described in ref. 21. The *w¹¹¹⁸* stock used in our experiments was the same stock used to create *w¹¹¹⁸;cry⁰* flies²¹. The *cry^b* line was a gift from P. Emery²². Our choice apparatus was based on the olfactory conditioning apparatus as described²⁹. Our two-coil system was based on the double-wrapped coil system described previously²⁴. We adjusted the current flowing through the coils so that the magnetic field intensity was no more than 5 G in any area along the tube. Coils were positioned 45° to the horizontal for experiments involving Canton-S, *w*;Canton-S, Berlin-K and Oregon-R flies. Coils were positioned parallel to the horizontal for all other experiments, because it produced a more robust response and eliminated a polar gradient; that is, there was no horizontal magnetic gradient, as the field was perpendicular to the T-port tubes. To assess the magnetoreponse of flies, we used a simple choice paradigm. Flies were placed in a glass vial containing

moistened Whatman paper and starved for 22 h before training. All experiments were performed between 8:00 and 12:00 EST. For each population of flies tested (100–150 flies per group), we calculated a preference index value on the basis of the equation: $(P_M - 0.5) / [(P_M + 0.5) - (2P_M \times 0.5)]$, in which P_M is the proportion of flies on the magnetic field side of the T-port. To test whether flies responded to the experimental magnetic field, we used either a Student's *t*-test to compare preference index values between trained and naive groups or a one-sample *t*-test to compare preference index values to zero (that is, preference index values expected with no response to the magnetic field).

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 S.M.R. and R.J.G. conceived the idea of using *Drosophila* to study magnetosensitivity; S.W. and R.J.G. conceived the idea of using appetitive conditioning to study magnetoresponses; R.J.G. designed the experimental apparatus; R.J.G., S.W., A.C. and S.M.R. designed the experiments and analysed the data; R.J.G. performed the experiments with help from A.C.; R.J.G., S.M.R., S.W. and A.C. wrote the paper.

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METHODS

Fly strains. Oregon-R, Berlin-K and w^{1118} stocks were provided by the Bloomington *Drosophila* Stock Center (product number 6326, 4269 and 8522, respectively).

Behavioural apparatus. The main chamber consisted of a training tube, a centre elevator section to transfer flies, and a two-tube choice point or T-port for testing the relative response of flies to a magnetic field (Fig. 1a). The training and T-port tubes were round-bottom polystyrene tubes and could be removed from the main section of the apparatus. The magnetic stimulus was delivered to the training and T-port tubes by placing the choice chamber apparatus inside an opaque housing box that contained the magnetic coil system. The box was constructed such that the main chamber could be placed between the two coils in either an upright position (as shown in Fig. 1a, top panel) or a horizontal position (as shown in Fig. 1a, bottom panel). The upright position of the chamber apparatus was used for training so that the tube could be placed in the centre of one coil, and the horizontal position of the apparatus was used to suspend the two tubes of the T-maze in the same area of the coil during test sessions. In this way, flies were subjected to the same light conditions and intensity of magnetic field during training and test sessions.

Each of the two coils was wrapped with two wires; the wires were wrapped in one direction on one coil and in the opposite direction on the other. Current flow through the coils produced a magnetic field in one coil (parallel current flow) but not in the other (opposite current flow). A double-pole, double-throw switch reversed the current flow in one wire loop but not the other, which allowed us to move the magnetic field easily from the right to the left side of the T-maze. A DC power supply with current and voltage controls was used so that we could change the intensity of the magnetic field produced by the coils. Each coil was mounted on a plastic track so that it could be positioned directly under the tubes (field perpendicular to the tubes), at the end of the tubes at a 45° angle (as shown in Fig. 1a), or at the end of the tubes (field parallel to the tubes). We used a magnetic field intensity of 5 G for our experiments, because it gave the most consistent naive response; decreasing to 1 G increased variability to the point that responses were no longer significant.

The housing box for the test or choice chamber was open on the top so that the chamber, regardless of position, could be illuminated by one ZooMed Reptisun 10.0 UVB fluorescent tube (F20T12) and one Agrobrite full spectrum fluorescent grow tube. Wavelengths entering the box were restricted by covering the top of the box with a long-wavelength filter that transmitted wavelengths of light >500 nm (Edmund Optics) or >420 nm or >400 nm (E400 and E420 from Gentex). Irradiance measurements (from an Ocean Optics USB 2000 fibre optic spectrometer) were taken from inside one arm of the T-maze portion of the choice chamber apparatus; thus, lighting conditions represent those experienced by flies while being either trained or assayed for sensitivity to a magnetic field.

Experimental procedure. For the training group, a population of 100–150 flies was loaded into the elevator section of the choice apparatus with an empty training tube facing one of the coils (Fig. 1a, top panel). Flies were transferred to the training tube for 2 min and then transferred back to the elevator and held for a 1-min rest period. The empty training tube was next replaced with a tube containing sucrose reinforcement and flies were allowed to feed for 2 min in the presence of a magnetic field. Flies were then transferred back to the elevator and held for 1 min while the coil system was turned off. During this time, the training tube was also removed, and two empty tubes were added to form the two arms of the T-port. The choice chamber was then positioned horizontally in the box (Fig. 1a, bottom panel). The coil system was turned on, and flies were transferred to the T-port, in which they were allowed to choose between the sides with or without a magnetic field. After 2 min, the two arms of the T-port were blocked and flies from each side were collected into separate empty vials and counted.

For the naive group, a second population of 100–150 flies was immediately loaded into the elevator section of the horizontally placed choice chamber and the coil system was turned on. After 1 min, flies were transferred directly to the T-port for 2 min.

Trained and naive groups were tested consecutively and with the magnetic field on the same side. This was done to control for the possibility that the choice behaviour of flies reflected a preference for one arm of the T-port and not a response to the magnetic field. As an extra control for side preferences independent of magnetic stimuli, we alternated the side of the T-port containing the field after each consecutive set of trained and naive flies (that is, trained and naive with magnet on the left side and then trained and naive with magnet on the right).