Zebrfish and medaka offer insights into the neurobehavioral correlates of vertebrate magnetoreception

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An impediment to a mechanistic understanding of how some species sense the geomagnetic field (“magnetoreception”) is the lack of vertebrate genetic models that exhibit well-characterized magnetoreceptive behavior and are amenable to whole-brain analysis. We investigated the genetic model organisms zebrfish and medaka, whose young stages are transparent and optically accessible. In an unfamiliar environment, adult fish orient according to the directional change of a magnetic field even in darkness. To enable experiments also in juveniles, we applied slowly oscillating magnetic fields, aimed at generating conflicting sensory inputs during exploratory behavior. Medaka (but not zebrfish) increase their locomotor activity in this assay. Complementary brain activity mapping reveals neuronal activation in the lateral hindbrain during magnetic stimulation. These comparative data support magnetoreception in teleosts, provide evidence for a light-independent mechanism, and demonstrate the usefulness of zebrfish and medaka as genetic vertebrate models for studying the biophysical and neuronal mechanisms underlying magnetoreception.

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he geomagnetic field (GMF) varies systematically across
the surface of the Earth in polarity (direction, North and
South), inclination (angle between field lines and the
Earth’s surface), and intensity, offering a spatial and directional
reference frame for orientation and navigation. Several animals
were reported to sense the Earth’s magnetic field (“magnetorec-
ception”, Fig. 1). Spiny lobsters may use the sense to return
home,5,6 migratory birds to find their destination,9 mole rats
show a preferred geomagnetic orientation when they build their
nest7,8, and cockroaches become more active in slowly oscillating
magnetic fields.4

Despite the widespread occurrence of magnetoreception across
different phyla (Fig. 1), the biophysical and neuronal mechanisms
underlying magnetoreception are poorly understood. Two main
hypotheses on the mechanistic basis of the magnetic sense exist.
(i) Magnetic fields can bias photochemical reactions involving
radical pairs (“radical pair hypothesis”). This might be physically
realized in cryptochromes of the retina, and translated into neu-
ronal signals under short-wavelength light (400–500 nm).10–12

Behavioral evidence exists for different species supporting such a
light-dependent sense13–16 and disruption of magnetoreceptive
behavior via weak magnetic fields in the radiofrequency range
seems to further support the radical pair mechanism17–19 (Fig. 1a). In addition, genetic manipulations in insects indicate
the involvement of cryptochromes in magnetoreception19,20 (Fig. 1a). This putative mechanism can work independently of light and is
consistent with behavioral data from several animals.21–23
Furthermore, altered orientation behavior after treatment with a
strong magnetic pulse indicates the involvement of magnetic
material in birds24–27. It is interesting to note that in some species
the two mechanisms seem to coexist and can detect different
parameters of the GMF.28–30

Candidate brain circuits of magnetoreception, such as the tri-
geminal brainstem complex in rainbow trouts31–33 European

Fig. 1 Behavioral evidence for magnetoreception across the animal kingdom. a Schematic summarizing the major experimental evidence for
magnetoreception in invertebrates and vertebrates as reported in the selected studies. The colored circles next to the references indicate whether
the behavioral experiments provide evidence for a light-dependent mechanism (i.e. consistent with the “radical pair hypothesis”, yellow), for a light-
independent mechanism (i.e. working under long-wavelength light or darkness, not consistent with a radical pair mechanism but consistent with the
“magnetite hypothesis”, black) or for the presence of a dual mechanism (yellow/black). The green shadow indicates genetic model organisms that are
accessible by whole-brain optical imaging. b Design of the present study performed on zebrafish and medaka at different developmental stages. Both
juveniles and sexually mature fish were studied by customized behavioral assays (“locomotor activity” for juveniles and “directional preference” for
sexually mature fish). Neuronal activation during the “locomotor activity” assay was mapped in medaka juveniles. dpf: days post fertilization.
robin39 and bobolink40, the visual system in mole rats41, and the ear and vestibular system in pigeons42,43, have been proposed. However, the tedious brain sectioning or invasive recordings necessary in these non-genetic and non-transparent vertebrate models pose serious challenges for unraveling the precise circuit underlying magnetoreception.

Due to their small size, relative transparency, as well as their amenability to genetic manipulations, the teleosts zebrafish (Danio rerio) and medaka (Oryzias latipes) can be ideal models for studying the biophysical and neuronal basis of vertebrate magnetoreception. Comparable features are found only in the nematode Caenorhabditis elegans, which however may employ different mechanisms for magnetoreception as compared to vertebrates44.

Recent reports have suggested that zebrafish are magnetoreceptive45-48, but no knowledge regarding the mechanism or the neuronal circuits is yet available. In this work, we sought to conduct a comparative study in two teleost models, to test (i) whether light-independent magnetoreception is present, and (ii) whether a behavioral assay could be established in young fish that is compatible with neuronal activity mapping across the whole-brain (Fig. 1b). We chose medaka for this comparison because, similar to zebrafish, it is transparent at early stages and easy to culture49, but has a less redundant genome50 which is ideal for genetic studies. Furthermore, in contrast to zebrafish, medaka seems to migrate between fresh and seawater, a behavior for which navigational capabilities are beneficial.

Our experiments show that the direction of the magnetic field influences the orientation of zebrafish and medaka adults even in the absence of visible light, suggesting the use of a light-independent sensing mechanism. Furthermore, we established a behavioral assay for juvenile fish, which indicates that magnetosensitivity is already present in young medaka, but not in young zebrafish. Brain activity mapping furthermore showed neuronal activation of the posterior lateral hindbrain in young medaka in response to stimulation with oscillating magnetic fields. This comparative study suggests that magnetoreception might be a common feature of teleosts and that zebrafish and medaka are attractive vertebrate models for future research on the biophysical mechanism and neuronal substrates of magnetoreception.

**Results**

**Zebrafish and medaka orient in response to the MF direction.** To assess magnetoreception in sexually mature fish, we employed a behavioral assay similar to Takebe et al.46, who showed directional preference of genetic cohorts of zebrafish in a magnetic field (MF) after release from the center of a circular arena (Fig. 2a). We chose an intra-subjective design in which each fish was tested twice, in two different magnetic conditions, obtained by setting the horizontal component of GMF 45° either towards East (NE) or West (NW), using two pairs of Helmholtz coils (Fig. 2a, b and Supplementary Fig. 1). This experimental design ensured that the current running through the coils was the same (but of opposite direction in the E-W coil pair) in both conditions. In distinction to previous studies in zebrafish46-48, the behavioral response could thus be confidently assigned to the change of the direction of the MF. In addition, we tested fish in presence or absence of short-wavelength light (Fig. 2c). For zebrafish, we then analyzed whether the change in bearing of each fish followed the 90° field deflection that we applied between conditions. Following Takebe et al.46, hearing (BE) was defined as the line between the center of the arena and the point where the fish first crossed a virtual circle (Fig. 2d, Supplementary Fig. 2). We found that under illumination with white light (WL, Fig. 2c) zebrafish significantly changed their bearing such that the distribution of angular differences between the two magnetic conditions (NE–NW) showed a mean axis which was consistent with the 90° deflection of the MF (Fig. 2e).

Next, we tested the fish under infrared illumination (IR, 1060 nm; Fig. 2c), which is not visible for many fish species, including zebrafish51. Besides avoiding potentially confounding visual cues, this experimental condition further aimed at assessing the presence of a light-independent mechanism of magnetoreception, postulated by the "magnetite hypothesis", discussed above. To avoid possible behavioral effects due to a change in illumination between acclimation and testing, one group of zebrafish was additionally acclimated to darkness for 60 min (D-IR group). This pre-adaptation period should further exclude any persisting photo-induced MF sensing, given the sub-second lifetime of the radical pairs in cryptochromes, which is assumed to determine the signaling state52 of updating neuronal processing during magnetoreception51,52. Moreover, behavioral experiments in birds tested under long wavelengths have suggested that exposure to darkness for an hour prior to testing is sufficient to prevent light-dependent magnetoreception52.

When we assessed the directional preference in this group of zebrafish, we found a significant change of the bearing, consistent with the 90° deflection of the MF (Fig. 2f). Interestingly, the distribution of the individual angular differences in the D-IR group seemed to be polar, and was significantly different from that observed in light (WL vs. D-IR, Watson U²; p < 0.05), indicating that two different mechanisms might be at play. Furthermore, when zebrafish were tested twice in the D-IR condition but without changing the direction of the MF, they showed a mean reorientation angle whose axis was consistent with a 0° deflection of the field (Supplementary Fig. 3). Without acclimation of the fish to darkness prior to testing under IR illumination (IR group), we observed a large scatter in the data resulting in a distribution that was not significantly clustered (Supplementary Fig. 4). This may have been due to the abrupt change in illumination between acclimation (in WL) and testing (under IR), which likely constituted a stress factor for the fish53,54.

When testing medaka under IR, we first noted that they exhibited a different swimming behavior. While zebrafish followed a rather linear trajectory towards the perimeter of the arena to then swim along the wall (thigmotaxis55, Supplementary Fig. 5, Supplementary Table 1, Supplementary Movie 1), medaka explored the arena by swimming in tight circles ("looping behavior") (Supplementary Fig. 6, Supplementary Table 1, Supplementary Movie 2), as also shown in rodent exploratory behavior56. To quantify whether the observed swimming behavior was influenced by the deflection of the MF (change in declination), we analyzed the spatial preference of medaka (SP) by determining the segment of the arena in which the fish spent the majority of time (Fig. 2g, Supplementary Fig. 7). We found that medaka adapted to darkness for 60 min and tested in IR (D-IR group, Fig. 2c) showed a significant axial change in spatial preference, compatible with the 90° deflection of the MF (Fig. 2h). Without acclimation to darkness prior to testing, we again observed a large variation in the directional preference change of an independent cohort of medaka (IR group, Supplementary Fig. 8).

Previous studies have shown that a directional preference relative to the MF exists within groups of zebrafish tested only once46,47. When we analyzed the initial trial for each fish in our experiments to assess the group response in a comparable fashion, we observed that zebrafish of the D-IR group (AB strain) showed a significant axial distribution in their directional preference (Supplementary Fig. 9a). This is similar to the result reported by Takebe et al. for the EKK zebrafish strain. The
medaka IR and D-IR groups, as well the zebrafish WL group, showed only an axial trend in their directional preferences (Supplementary Fig. 9b–e).

Taken together, these data indicate that both zebrafish and medaka change their directional preference with respect to the direction of an applied MF also in the absence of visible light. The results suggest that a light-independent mechanism for magnetoreception may be a common trait of teleosts. The results do however exclude that teleost fish might be able to employ also a light-dependent mechanism, if short-wavelength light is available.

Young medaka change their locomotor activity in oscillating MF. Next, we asked whether magnetosensitivity is present already in younger fish, which would be ideal subjects for subsequent studies aimed at understanding the molecular and neuronal mechanisms of magnetoreception because of their small size, transparency, and amenability to genetic modifications. We thus set up a magnetic stimulation paradigm designed to maximize the likelihood of detecting MF-dependent behavioral and neuronal responses. In this assay, each fish was allowed to explore an unfamiliar circular arena in both of the following conditions for 120 s each: static background MF (sham) and slowly oscillating MF. Each fish was tested in randomized order under both conditions that differ by a 90° deflection of the MF. For zebrafish, the bearing (BE) is determined as the angle from the center of the arena to the point where the fish crosses a virtual circle (radius of 6 cm). The change in preferred direction, i.e. the angular difference between the two conditions (NE−NW) is calculated for each fish. For medaka, the spatial preference (SP) is assessed during the second minute after release. Distribution of the angular differences for medaka (Cab strain) in D-IR. Each dot in the circular plots represents the individual angular difference, the arrow indicates the mean vector, double arrows indicate axial symmetry computed by doubling the angles. The number of fish, the mean angle with the 95% confidence interval (CI), and p values for the Rayleigh test for circular uniformity as well as the V-test (testing for circular uniformity against the alternative hypothesis of a mean angular difference of 90°) are reported. Statistical tests were performed on the axial data when such symmetry was observed.

**Fig. 2** Adult zebrafish and medaka orient with respect to the direction of a magnetic field also in absence of visible light. a-c Schematics of the experimental setup and procedure. a, b In the experiment, fish are automatically released from the center of an arena and Helmholtz coils are used to deflect (change in declination) the GMF 45° towards West (NW, red) or East (NE, purple). c Before the experiment, fish are acclimated in white light (WL) or in darkness (D) for 60 min and tested under WL or infrared illumination (D-IR). Each fish is tested in randomized order under both conditions that differ by a 90° deflection of the MF. d For zebrafish, the bearing (BE) is determined as the angle from the center of the arena to the point where the fish crosses a virtual circle (radius of 6 cm). The change in preferred direction, i.e. the angular difference between the two conditions (NE−NW) is calculated for each fish. e, f Distribution of the angular differences for zebrafish (AB strain) in WL and D-IR. g For medaka, the spatial preference (SP) is assessed during the second minute after release. h Distribution of the angular differences for medaka (Cab strain) in D-IR. Each dot in the circular plots represents the individual angular difference, the arrow indicates the mean vector, double arrows indicate axial symmetry computed by doubling the angles. The number of fish, the mean angle with the 95% confidence interval (CI), and p values for the Rayleigh test for circular uniformity as well as the V-test (testing for circular uniformity against the alternative hypothesis of a mean angular difference of 90°) are reported. Statistical tests were performed on the axial data when such symmetry was observed.
Juvenile medaka increase their locomotor activity when stimulated with a slow oscillating MF in white light. To control for non-magnetic effects such as electrical noise (Methods), we used double-wrapped Helmholtz coils in East−West axis with respect to the static background GMF (Fig. 3a, b). To test this, we calculated the cumulative frequency of complete turns (90°) that occurred during exploration. While no effect was observed in zebrafish (Supplementary Fig. 10d, e), we found that during the 30 s immediately before and after the change in condition, medaka performed less complete turns in the soMF condition (Fig. 3d). When looking at group 1 and group 2 separately, a trend towards a decrease in turns could be observed in group 1 and a significant decrease between sham(t1) and soMF(t1) (Supplementary Fig. 10f).

Altogether these data suggest that juvenile medaka alter their exploratory behavior in response to a continuously changing MF, thus demonstrating that magnetosensitivity is present already in these young fish.
Mapping brain regions activated by oscillating MF in medaka.

We were next interested in searching for brain areas that were differentially activated by stimulation with soMF during exploratory behavior. To this end, we chose a brain mapping technique based on immunohistochemical detection of the level of phosphorylated ERK (pERK), which shows a high degree of correlation with neuronal activity\(^{59,60}\). This approach has recently been employed to detect neural substrates of specific behaviors in freely swimming zebrafish\(^{60}\).

Conveniently, whole-mount brain analyses are possible in medaka juveniles because of the small size and the relative transparency of their brains. Using the same assay as shown in Fig. 3, a cohort of juvenile medaka was stimulated for 10 min while freely exploring a test arena during soMF or with the double-wrapped Helmholtz coils run in sham mode as control (same currents but in antiparallel sense, so no resulting MF).

Immediately after the experiment, fish were preserved in 4% paraformaldehyde (PFA) for subsequent immunostaining against pERK (marker of activated neurons) and tERK (total ERK, with a broad expression providing anatomical information and a reference for signal normalization\(^{60}\)).

We quantified the pERK fluorescent signals in several brain regions that have previously been suggested to be involved in magnetoreception in various species, and that were readily discernible in our stained specimens (Fig. 4a). These include: (1) the olfactory epithelium (oe), suggested to contain magnetoreceptor cells in rainbow trout\(^{61}\); (2) the pineal gland (pg), (3) the habenula (hb), found responsive to MFs in rats and birds\(^{62}\), the (4) lateral cerebellum (lcb) and (5) hindbrain (lhind), responsive to constantly changing MFs in homing pigeons\(^{42,63}\) and European robins\(^{39}\).

While our analysis of fish stimulated with soMF failed to reveal changes in pERK signal in the olfactory epithelium, pineal gland, habenulae, and cerebellum (Fig. 4b–f respectively), it showed a significant increase of activity in the lateral hindbrain (lhind, Fig. 4f). This result was replicated in an independent cohort of fish (Supplementary Fig. 11). Even though both the hindbrain and cerebellum are generally involved in controlling motor coordination and locomotion in fish\(^{64,65}\), increased activation was observed only in the hindbrain. Taken together, these results identify the lateral hindbrain as a candidate brain region that may be involved in the magnetosensitivity observed in medaka.

Discussion

In this study we adapted and developed specific behavioral assays and analyses to assess magnetoreception in two genetic teleost models, zebrafish and medaka, at different times during their life cycle. To assess magnetoreception in sexually mature fish, we chose an intra-subjective design, where each fish was tested twice under two different directions of the magnetic field. The directional preference assay that we conducted in independent cohorts of sexually mature fish (Figs. 1b and 2) showed that both species are magnetoreceptive also in the absence of visible light. However, we cannot exclude the possibility that a light-dependent mechanism also exists, one that may work in addition or in parallel whenever short-wavelength light is available. Given the evidence for the coexistence of two mechanisms that has been
reported for several species. Although a larger number of observations may have revealed a stronger clustering of the directional preference of the group, as has been previously reported for zebrafish, our result may also be explained by the conditions in which the directional preference assay was conducted. In particular, testing fish in isolation and with no spatial cues but the MF prevents them from referencing and adjusting their individual directional choice to other spatial information and to the behavior of conspecifics, conversely to what occurs in nature within schools of fish. Behavioral tests on cohorts of fish tested in groups and/or with additional spatial cues present may thus be informative also with respect to the ecological relevance of the observed magnetoreception for navigation purposes.

The “locomotor assay” we developed (Figs. 1b and 3) further provided evidence that already at young adult stages, medaka respond to weak and slowly oscillating MFs by changing the velocity and the structure of exploratory swimming. Interestingly, comparable hyperactivity has also recently been reported for insects exposed to oscillating MFs. An increase in locomotion and feeding rate was also observed in several teleosts during natural geomagnetic disturbances. With respect to young zebrafish however, the inability of our assay to detect a behavioral effect leaves it an open question whether magnetoreceptive behavior occurs later during development, or whether a different assay is needed to detect this behavior in young zebrafish.

Furthermore, the design of the behavioral assay and the small size of juvenile medaka allowed us to readily search for related brain activation patterns using whole-mount histological techniques. We identified the lateral hindbrain as a candidate region that was differentially activated during soMF-induced changes in exploratory behavior (Fig. 4D). By homology to zebrafish and other vertebrates’ functional anatomy, the lateral hindbrain of medaka is likely to process inputs from cranial or peripheral sensory systems, such as the vestibular, lateral line, and trigeminal ganglia. As discussed, an involvement of both vestibular and trigeminal systems has been proposed in vertebrates.

Live neural recordings during repeated presentations of magnetic stimuli with a systematic change of parameters, together with analyses of the connectivity patterns, will be useful to expand on the whole-mount pERK analysis performed in this study. This may help to disentangle stimulus-related neuronal activation patterns from brain activities more closely linked to magnetoreceptive behavior and to possibly also trace connections back to the candidate magnetoreceptor cells. The behavioral assays combined with neuroimaging may also provide a useful readout for forward genetic screens.

To conclude, we provide evidence for a mechanism of magnetoreception in the teleost fish zebrafish and medaka that is independent of visible light. We developed a simple assay to measure magnetoreceptive behavior already in juvenile medaka and identified the lateral hindbrain as a candidate brain region involved in magnetosensitivity using a histological brain mapping technique. These data show that the genetic and optical accessibility of these laboratory teleosts makes them attractive models for in-depth follow-up studies to uncover the biophysical sensing mechanism and neuronal computation underlying magnetoreception.

Methods

Directional preference assay in adult zebrafish and medaka. Adult zebrafish (AR strain) and medaka (Cah strain) were used in this study (see Supplementary Table 2 for details). The fish were fed with Artemia twice per day and kept in a standard 14/10 h light/dark cycle. Zebrafish were offspring from a single parent couple and therefore genetically similar. All animal experiments were approved by the government of Upper Bavaria and were carried out in accordance with the approved guidelines. We took particular care to minimize any cues by installing the coil set-up in a dedicated laboratory space at the Institute of Zoology at University of Hohenheim or at the paleomagnetic laboratory “Niederpfahl” of the Ludwig-Maximilian University (LMU). The test arena (Fig. 2a) was a spatially uniform glass petri dish (O 17 cm, H 3 cm) located inside an opaque (Black postcard hardboard, TB4, Thorlabs) placed on a white table and covered with a black curtain (Blackout fabric BK5, Thorlabs) blocking any ambient light from reaching the test arena. In addition to the black curtain covering the arena, other light sources in the room were switched off or covered with black tape such that all visual stimuli were abolished in the IR and D-IR conditions. The table was located in the center of two pairs of Helmholtz coils. The coils were used to deflect the horizontal component (H) of the GMF in the room 45° towards either the East (NE) or West (NW) in a configuration in which equal amounts of current were flowing through the coil in both conditions (Supplementary Fig. 1). The coil pair in the East–West orientation generated the directional deflection of the MF, while the intensity was adjusted by applying a current in the North–South orientation (Supplementary Fig. 1), controlled with a compass and a magnetometer. The horizontal (H) and the vertical (V) component thereby remained at GMF strength ([H] 23.3±1.5 μT, V 40.5±1.5 μT, total 50.6±1.5 μT, inclination 62.6°). The environment was kept unchanged between the two conditions for each trial, except for the change in the horizontal component of the local GMF at the center of the transparent plastic circular cylinder (O 6 cm) was moved up by an automatic lifting mechanism to release the fish. A custom-made IR illumination table, consisting of an array of IR LEDs (1060 nm, ELD-1060-525, Jenoptik, Germany, with a diffuser on top) was placed underneath the arena. For the WL experiments, a WL ring illumination (LED centered above the petri dish) was used to provide a broad range of illumination including IR was used for video recording (Sony DCR-HC23E). The IR illumination was switched on during all experiments while videos were captured at 25 frames per sec (FPS). During the acclimation phase (Fig. 2c), fish were kept individually for 1 h in small tanks placed inside boxes made of opaque material (Black postcard hardboard, TB4, Thorlabs) and illuminated from another light source. The direction of the MF (NW or NE) was set before fish were placed individually in the inner cylinder at the center of the test arena and left there for 20 s before being released. The swimming trajectories were recorded for 1 (in the case of zebrafish) or 2 (in the case of medaka) minutes before the fish were removed from the arena and placed back to the individual box. Fish were tested randomly in the NW or NE condition and left to rest for ~45 min before being tested again in the opposite magnetic condition (or the same magnetic condition in case of the 0° experiment, Supplementary Fig. 3). Fish that were tested after acclimation in darkness were transferred to the test arena under red light illumination.

By measuring the orientation of the nose of the fish the behavior was performed automatically with a custom written routine in Matlab (MathWorks, MA, USA). To assess the bearing (BE) of individual fish, the program determined the point at which the nose of the fish was crossing the virtual circle of radius 6 cm (Fig. 2d). The bearing was then defined as the angle from the center of the arena (the crossing point, relative to the magnetic North). For assessing the spatial preference (SP), the arena was divided into 12 segments and SP was defined as the segment in which the fish (using the centroid) spent most of the time during the second minute after release. SP could still be determined in case the automatic release mechanism failed. Manual corrections were made in a few cases (4 out of 87) in which the tracking software could not correctly track the fish for the entire time of the experimental run. The difference in the preferred segment between the two conditions (NE, NW) was converted to an angle (in steps of 3°) for each fish (Fig. 2g). The directional preference of the group (Supplementary Fig. 9) was assessed by analyzing the first trial of each fish normalized to the geomagnetic field. The orientation of circular statistics was performed using R (R Development Core Team, 2011). Computing Services). The distribution of angular differences (either polar or axial) were assessed by the Rayleigh test for clustering of data as well as comparing the expected mean of 90° by inspecting the 95% confidence interval and using the V-test, which tests for uniformity against an alternative hypothesis of a distribution with a specified mean (in our case 90°). Directional preference results were represented in an angle (in steps of 3°) for each fish (Fig. 2g). The directional preference of the group (Supplementary Fig. 9) was assessed by analyzing the first trial of each fish normalized to the geomagnetic field. The distribution of angular differences (either polar or axial) were assessed by the Rayleigh test for clustering of data as well as comparing the expected mean of 90° by inspecting the 95% confidence interval and using the V-test, which tests for uniformity against an alternative hypothesis of a distribution with a specified mean (in our case 90°). Directional preference results were represented in an angle (in steps of 3°) for each fish (Fig. 2g).
Swimming strategy for adult medaka in light and darkness. To assess the swimming strategy of sexually mature medaka in IR compared to WL (Supplementary Fig. 6, Supplementary Table 1) one group of fish were tested in both light conditions. Eleven fish of mixed gender of the Cab strain, aged ~6 months, were observed individually for 2 min in a circular arena (15 cm diameter). Six individuals were tested first in IR, and then in WL, while the remaining fish were tested first in WL and then in IR. The arena was illuminated from below with a ring of LEDs (1060 nm) and a WL illumination table. The IR source was always turned on, while the illumination table was switched on only for the WL trial. Whatman paper underneath the arena created a homogeneous white background. Fish were imaged for 2 min at 20 FPS from above with a near-infrared sensitive camera (Ximea MQ0135G-E2, Germany). The whole setup was placed within a black box made of carbon not transparent to light (Black hardboard TB48, Thorlabs, USA).

The natural swimming behavior in the two illumination conditions were assessed with Ethovision software (Ethovision XT, Noldus, the Netherlands).

Continuous looping behavior was assessed through the rotation parameter available within the software, where 720° rotations of the fish heading were counted (using 0° threshold, not accepting any backwards movement, with a minimum distance of 2.5 cm). For statistical analysis, the frequency of the behavioral data was assessed in GraphPad Prism 6, calculating Shapiro–Wilk normality test, KS normality test, and D’Agostino & Pearson omnibus test. Only in case the data showed a normal distribution according to at least two out of the three tests, a t-test was used, otherwise nonparametric tests were employed. Plots were generated in GraphPad Prism 6.

Locomotor activity in juvenile fish. Zebras of the AB strain and medaka of the Cab strain were grown at 28°C with a 14:10 h light:dark cycle. From day 5 onwards, zebras larvae were raised in the fish facility under standard conditions. At 13 dpf (day post fertilization) the fish were starved for 24 h prior to the experiment. Medaka juveniles were tested within 5 days after hatching (between 10 and 12 dpf). See Supplementary Table 4 for details. A transparent circular arena (Ø 6 cm) was placed on a transparent glass table above a homogeneous LED WL source (900 nm, LED Drawing table, 22075 663, 420–700 nm), in the center of a pair of double-wrapped Helmholtz coils.39,59 These coils work either in sham-mode or in field-mode such that the power delivered from the power supply is equal in both conditions, and thus not producing noise depending on the amount of delivered power. In the sham-oscillatory field condition (control) the currents were alternating in the same frequency as in the experimental condition, i.e., the MF was generated when the current ran in parallel, while in sham mode the currents ran in opposite directions within the coils resulting in no net MF at the test arena while producing the same electric noise and ohmic heating (which was however minimal). An oscillating MF (sinusoidal with peak value 40 μT oscillating at 1 Hz) was applied in the East–West direction (Fig. 3), resulting in a continuous change in both direction and intensity of the field. The swimming of the fish in the arena was imaged with the Ximea MQ003MG-CM camera from above (the custom-made setup is depicted in Supplementary Fig. 12). The fish were introduced in the arena containing fresh water (same as the tank water in which the two species are raised and cultured) with the oscillating field either in the magnetic (soMF) or in sham mode (sham). After 2 min, the stimulation was changed to soMF (group 1) or sham (group 2), respectively, and the fish were observed for another 2 min.

Analysis of the locomotor activity was performed using Ethovision software (Ethovision XT, Noldus, the Netherlands). Velocity and number of turns were computed automatically and in the case manual corrections to the swimming trajectories were necessary, this was done without knowledge of the experimental condition. Turns were defined as 90° rotations of the fish heading (using 0° threshold, not accepting any backwards movement). Fish that were inactive (mean swimming velocity below 0.01 cm s⁻¹) were excluded from the analysis. Behavioral data were tested for whether they were consistent with a normal distribution using three tests for normality (Shapiro–Wilk, KS, and D’Agostino & Pearson omnibus normality test as implemented in GraphPad Prism 6). If at least two out of three tests were consistent with a normal distribution, a t-test was used for statistical analysis. If the parametric tests were employed, respective corrected parameters, refer to Supplementary Tables 4, 5, and 6 for a summary of the statistical analysis. Plots were generated in GraphPad Prism 6.
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Author contributions

A.M. and A.L. designed, performed, and analyzed all the experiments. S.H.K.E and D.S. designed and built the coil systems for the experiments in sexually mature fish and performed preliminary experiments. S.H.K.E. contributed to the design of the experiments conducted on juvenile fish and built the double-wrapped Helmholtz coils used for this experiment, performed the experiments on the sexually mature fish together with A. M. and A.L. and gave feedback on the manuscript. M.C. wrote the algorithm used for automated tracking and analysis of the bearing of the adult fish and gave feedback on the statistical analysis. W.W. supported the project and provided feedback. M.W. contributed to the design of the adult experiments, supported the project and provided detailed feedback on the manuscript. A.L. conceived and generated the illustrations. G.G.W. designed, coordinated, and supervised the study. A.L., A.M., and G.G.W. wrote the manuscript.

Additional information

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