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The light-independent locomotion response to a static magnetic field in *Xenopus* tadpoles

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Increasing numbers of behavioral observations have shown that many organisms can sense magnetic fields in the environment. However, the underlying mechanisms of magnetosensation remain elusive, and a major problem is the lack of an animal model convenient for detecting magnetic responses, especially in vertebrates. We have previously reported that the physical performance of mice decreased after 1 month of exposure to a hypomagnetic field, which probably includes a light-independent mechanism. In this study, we investigated the effects of a static magnetic field on the locomotion of *Xenopus* tadpoles (stage 48) using a real-time recording system. Because it is speculated that the photosensing system is related to magnetosensation, we triggered the tadpoles to swim by a 30 s light pulse after a 5 min adaptation in the dark and measured the swimming activities of the tadpoles under static magnetic field exposure in both the dark and bright conditions. Compared to the sham control treatment, the presence of a magnet inhibited the movement of the tadpoles under both bright and dark conditions, as shown by reductions in swimming distance, speed, and counts of path adjustment. Moreover, the directional preference for path adaptation was altered when swimming in the dark. These results suggest that tadpoles in the dark can exert a rapid locomotion response to changes in the environmental magnetic field, providing evidence for an intrinsic, light-independent rapid magnetoresponse pathway in tadpoles. In addition, this rapid vertebrate locomotion assay paradigm will be a practical tool to facilitate further investigation of the mechanisms of biomagnetic effects.

KEYWORDS

magnetoresponse, locomotion, light independent, xenopus tadpole, magnetic field, path adjustment, direction preference

1 Introduction

How organisms perceive and respond to magnetic fields is a fundamental question that remains to be answered. Accumulating behavioral evidence has shown that many organisms can sense environmental magnetic fields. The movement of magnetotactic bacteria and the geomagnetic navigation of insects, fish, and migratory birds [1] show that organisms can use geomagnetic field (GMF) information to coordinate their locomotion. Our recent studies have shown that elimination of the GMF, *i.e.*, the hypomagnetic field (HMF), induces changes in the circadian rhythms of drinking behavior, physical performance, and anxiety-related behavior in mice, accompanied by metabolic changes [2–5], suggesting that the GMF is required for the maintenance of the normal animal behavior. Although most studies have focused on the effects of GMF direction on animal behavior [6–9], accumulating evidence has also been shown that animal behavior can respond to changes in the intensity of the GMF [10–13]. However, the understanding of the mechanism of magnetoresponse in relation to magnetic field intensity is still very limited.

It has been reported that the photosensing proteins, cryptochromes (CRYs), are involved in animal magnetoreception. Ritz et al. indicated that the photoreceptor cryptochrome is part of the magnetoreception system [14]. Gegeer et al. provided the first genetic evidence for a CRY-based magneto-sensitive system in *Drosophila* [15]. Foley et al. used a transgenic approach to demonstrate that human CRY2, which is highly expressed in the retina, can function as a magnetosensor in the magnetoreception system of *Drosophila* [16]. Wan et al. showed that CRY1 from *Drosophila* and monarch butterflies plays a key role in magnetosensation in a light-dependent manner [17]. However, because ultraviolet (UV)-A/blue light is required for photosensing CRYs to form magnetically sensitive radical pairs [16–19], GMF driven navigation of animals at night could not be fully explained by the light-dependent mechanism.

It has also been reported that non-photosensing proteins/particles are involved in magnetosensation. Magnetite is a commonly recognized magnetoreceptor that can explain orientation-dependent magneto-sensing [20–25]. Structural proteins and enzymes may also respond to the HMF. We have found that exposure to the HMF disrupts the *in vitro* assembly of cytoskeletal proteins, tubulin, and actin [26, 27], accelerates the denaturation of Cu, Zn-Superoxide Dismutase (CuZn-SOD) [28], and increases the activity of lactate dehydrogenase (LDH), a key enzyme in glycolysis [5]. Therefore, it is worth investigating whether other light-independent factors besides CRY and CRY-related signaling pathways, are involved in the general response of animal behavior to the environmental magnetic field.

The lack of a simple and robust *in vivo* magnetosensation model suitable for genetic modification and behavioral testing is a significant limitation to the study of magnetosensation. We have previously monitored mouse behavior in an HMF and found that a 3 days exposure causes anxiety [29], and a 1-month exposure can reduce physical performance [3, 4]. However, these HMF effects are accumulative, require long-term exposure, and do not lend themselves to the detection of rapid intrinsic magnetosensing behavior. For the behavioral effects observed in other applied magnetic fields, a real-time behavioral recording system for immediate monitoring and analysis under specific magnetic field conditions is lacking [6, 10, 25, 30–35].

The African clawed frog (*Xenopus laevis*) is a commonly used vertebrate model. *Xenopus* embryos are a suitable material for functional analysis. They develop rapidly *in vitro*, are easy to transgene and detect their effect, and it is easy to obtain large numbers of embryos. The small size of the tadpoles also allows simultaneous treatment and behavioral observation of multiple individuals in a limited workspace. We have previously observed that *Xenopus* embryos in an HMF exhibit abnormal embryonic development [36], indicating that they are good candidates for the examination of the effect of environmental magnetic fields and are suitable for further investigation of the genetic basis of bio-magnetic effects.

In this study, we set up a suitable paradigm for analyzing the locomotion of the *Xenopus* tadpole to investigate its rapid response to an applied static magnetic field (SMF) under both dark and bright conditions. Because the magnetoresponse in animals is thought to be closely related to the photosensing system, tadpoles at stage 48 with distinct photo-motor sensations were used. Tadpoles were exposed to a medium-intensity (1mT - 1T) SMF generated by commercially available neodymium iron boron (NdFeB) magnets, commonly used in magnet therapy and mechanistic studies in animals [37, 38]. With the light-dependent hypothesis of magnetosensation, magnetic responses should be detectable only under the light condition rather than in the dark. While with the light-independent hypothesis, the magnetoresponse should be observed in both the light and dark conditions. Furthermore, if the signaling of magnetoresponse is synergistic with the photosensing system, the light-triggered response, *e.g.* photo-motor sensations, would be affected (amplified/weakened) by the magnetic stimulation, *i.e.*, exhibiting magnetoresponse. Using this experimental system, we aimed to characterize the behavioral response of tadpoles to the SMF and evaluate the role of light in the magnetic response of tadpoles. We find a light-independent rapid locomotion response to the static magnetic field, which provide clues to the mechanism of the magnetoresponse with a practical *in vivo* paradigm for further studies.

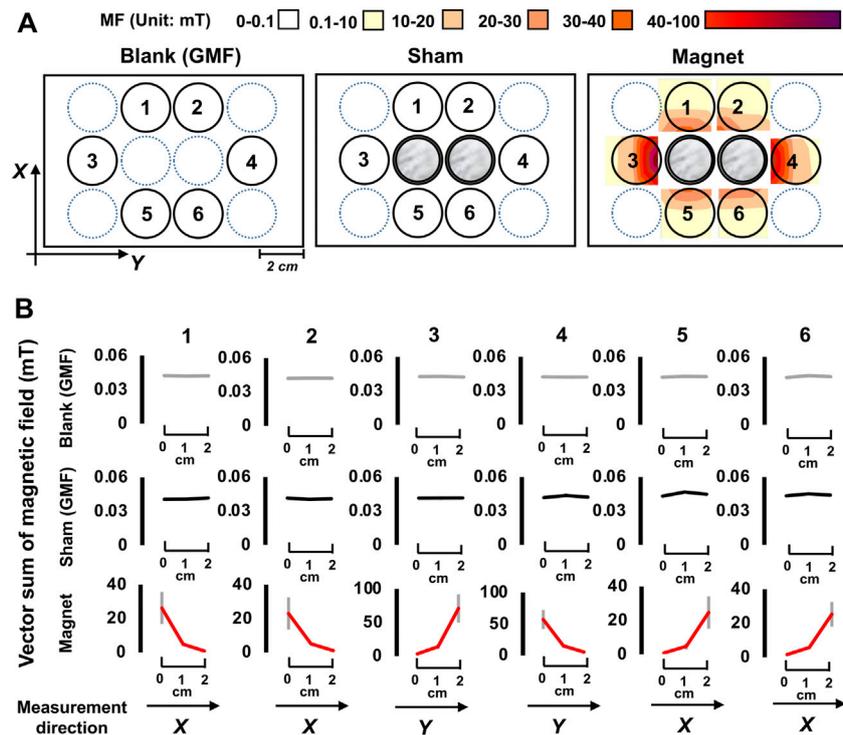


FIGURE 1

The experimental setup and magnetic field conditions. (A) The distributions of the magnetic field (vector sum) at the bottom plane of a 12-well plate for the blank, sham, and magnetic treatments are shown. Tadpoles were placed in wells with Arabic numerals 1 to 6. The central two wells (in gray) were the positions for magnetic (magnets) or sham (nickel coins) treatments. Black arrows indicate direction of the magnetic field. X-axis, South to North; Y-axis, East to West, MF, magnetic field. (B) The distribution of magnetic field vector sum along the X- or Y-axis, data are shown as mean \pm standard deviation (SD) of the vector sum in each test well.

2 Material and methods

2.1 *Xenopus* tadpole preparation

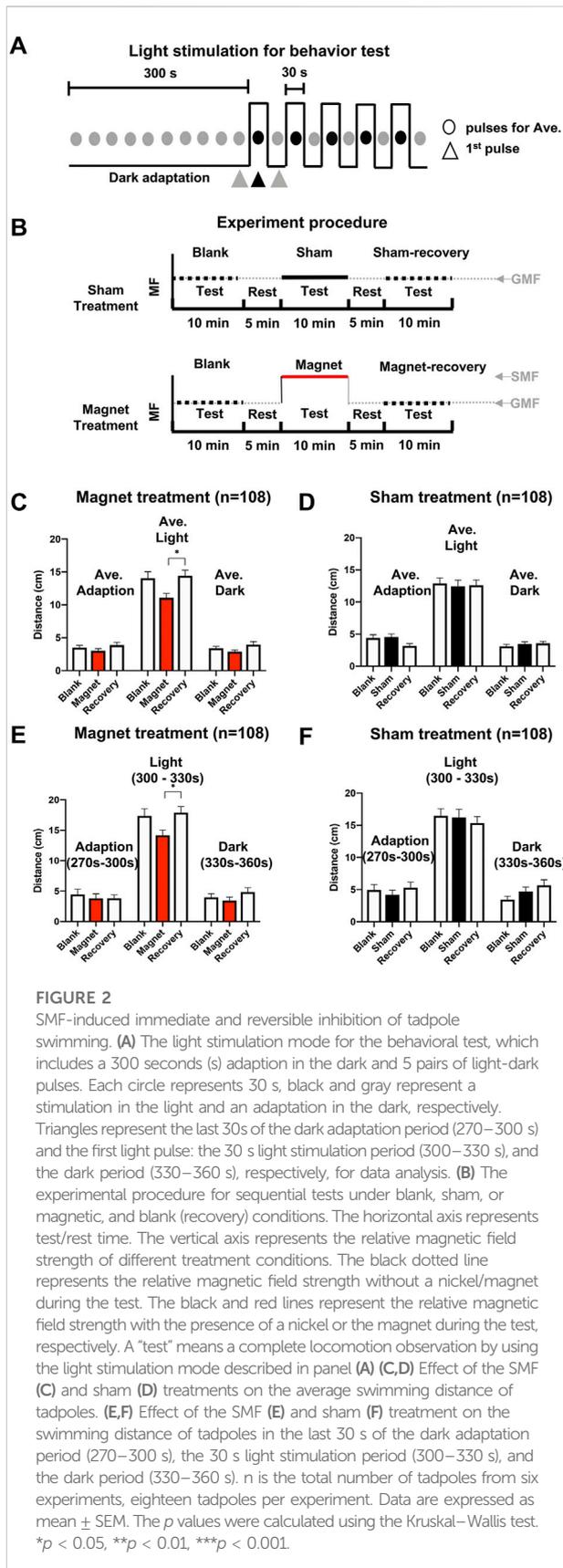
Xenopus tadpoles (stage 48) were used in this study. *Xenopus* embryos were prepared for *in vitro* fertilization according to a previously published protocol [39]. Embryos were cultured with $0.1 \times$ MMR solution (10 mM NaCl; 0.2 mM KCl; 0.2 mM CaCl_2 ; 0.1 mM MgCl_2 ; 0.5 mM HEPES, pH 7.4). Progression of development was monitored at a temperature of 14–24°C. Embryonic stages were determined as previously described [40, 41]. All animal experimental procedures were performed according to institutional ethical guidelines (grant No. SYXK2014-30).

2.2 Magnetic field conditions

Tadpoles were tested in 12-well plates containing 3 ml of $0.1 \times$ MMR solution. As shown in Figure 1, six wells around the central zone were selected as test arenas. Two stacks of NdFeB permanent magnets and nickel coins, both wrapped in tin foil,

were placed in the two central wells for the SMF (magnetic) and sham treatment, respectively. No objects were placed in the blank GMF control. The environmental magnetic field (MF) on the blank and sham control plates was measured using a 3-axis fluxgate magnetometer constructed by the National Space Science Center (NSSC), of the Chinese Academy of Sciences (CAS). The magnetic properties of the SMF were measured using a Teslameter (HTME tech, HT20, Shanghai, China).

In the sham treatment, tin-foil-wrapped nickel coins of the same size and appearance as the tin-foil-wrapped magnets were used to control non-magnetic factors. The presence of the sham nickel stacks had no effect on the MF. The MF intensity in the test arenas under the blank and sham conditions was 0.042 ± 0.001 mT and 0.043 ± 0.005 mT, respectively, corresponding to the GMF level (0.04–0.06 mT). Under the SMF condition, it exhibited a high to low gradient along the central-to-peripheral direction. The gradients were at the same level in arenas 1, 2, 5, and 6 (0.1–30 mT), and became stronger in arenas 3 and 4 (0.1–80 mT) (Figure 1). The directions of the magnet-generated MF in the test arenas were largely downwards and northwest (in contrast to the blank and sham, that were downwards, and northeast).



2.3 Locomotion test

Locomotion of the stage 48 tadpoles was monitored using a Zebralab system (Viewpoint, France) in six test wells of the plate, with one tadpole per well. The temperature was constant at around 25°C. The length of a tadpole is about 15mm, and the width is about 2.5mm, the body size accounts for about 1/10 volume of the test well, so tadpoles have ample space for movement. The tadpole locomotion test lasted a total of 10 min: a 5 min dark adaptation followed by 5 min alternating light (full spectrum of visible light, 900 lx) pulse stimulation with 30 s light on and 30 s off (dark) (Figure 2A). Prior to each test, tadpoles were transferred to the test arenas for a 5–10 min accommodation period. Swimming tracks were recorded with an infrared camera, especially under dark conditions.

In the first experimental procedure, a single tadpole was tested sequentially under different conditions, blank-sham or magnet-blank (recovery) (Figure 2B), and the average swimming distance in the dark and light was calculated. Additionally, the swimming distance was calculated in the last 30 s of the dark adaptation period (270–300 s), first 30 s light stimulation period (300–330 s), and dark period (330–360 s). The blank tests before the treatments served as a quality control to ensure that the tadpoles exhibited normal photomotor responses (Supplementary Figure S1).

For the second experiment, the same batch of tadpoles was tested under the sham and magnetic conditions sequentially (Figure 3B). Data from the last 30 s of the dark adaptation period (270–300 s) and the first 30 s of the light stimulation period (300–330 s) were collected for each test.

2.4 Locomotion indexes

Locomotion indexes for movement intensity and path adaptation were determined using Viewpoint tracking and locomotion analysis software (Viewpoint, France). The intensity indexes include moving distance, speed, and active duration (instantaneous speed ≥ 0.25 cm/s). Indexes for path adjustment included path angle adjustment and the number of adjustments at each angle.

2.5 Data analysis

All experiments were performed independently at least six times, with 12–18 tadpoles per experiment. Tadpoles showing no large movement during the blank test were excluded. Means were calculated with all tadpoles from the experiments, data are expressed as mean \pm standard error of the mean (SEM) unless otherwise stated. The

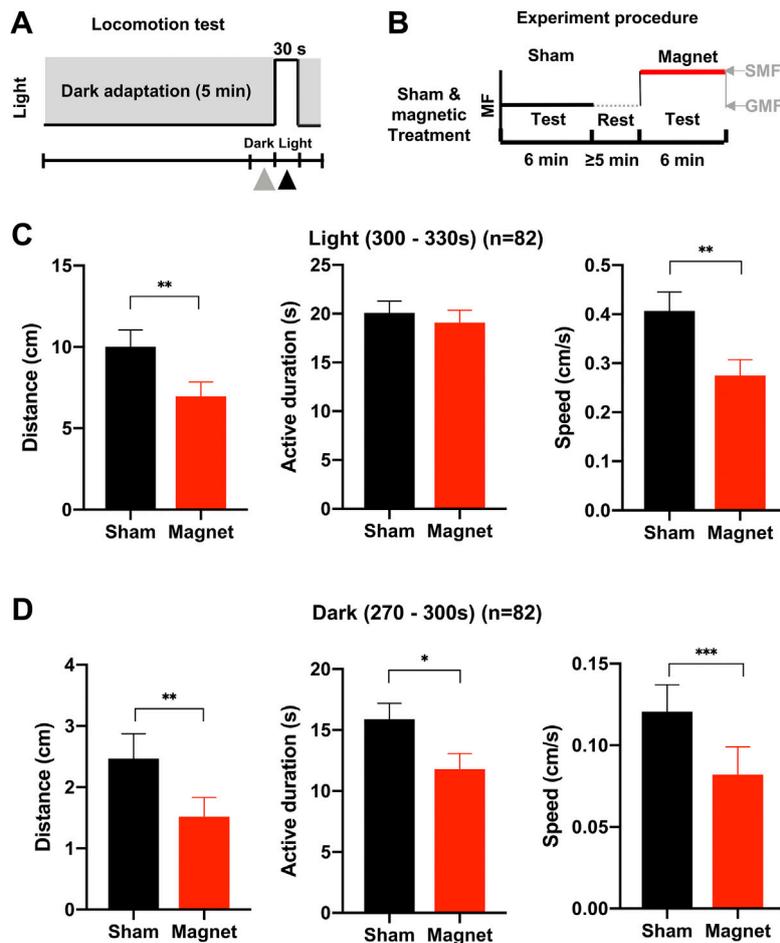


FIGURE 3

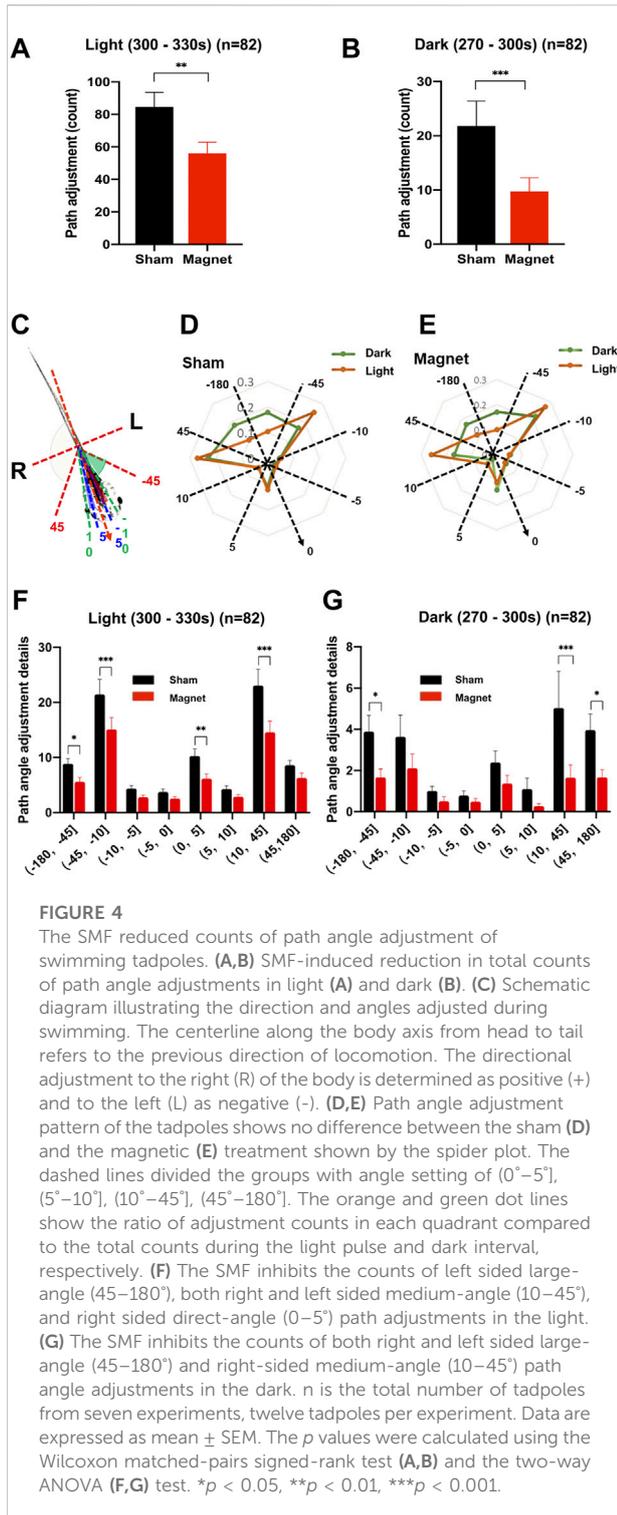
The effect of the SMF on the swimming behavior of tadpoles differs between light and dark conditions. (A) The light stimulation mode for the behavioral test. The triangles represent the last 30s of the dark adaptation period (270–300 s) and the first light pulse (the 30 s light stimulation period (300–330 s), respectively, for data analysis. (B) The experimental procedure for sequential tests under sham and magnetic conditions. The horizontal axis represents test/rest time. The vertical axis represents the relative magnetic field strength of different treatment conditions. The black dotted line represents the relative magnetic field strength without a nickel/magnet during the test. The black and red lines represent the relative magnetic field strength with the presence of a nickel and the magnet during the test, respectively. A “test” means a complete locomotion observation by using the light stimulation mode described in panel (A) (C,D) Effects of the SMF treatment on locomotion indexes of tadpoles in the dark (C) or in the light (D). SMF inhibits tadpole swimming distance and speed in the light condition, and the swimming distance, active duration, and speed in the dark. *n* is the total number of tadpoles from seven experiments, twelve tadpoles per experiment. Data are expressed as mean ± SEM. The *p* values were calculated using the Wilcoxon matched-pairs signed-rank test. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

Kruskal–Wallis test was used for the mean comparison of nonparametric data between three or more groups, like the distance in Figures 2C–F. The Wilcoxon matched-pairs signed-rank test was used for comparison of nonparametric data between two groups, such as swimming distance, active duration, speed, and path adjustment (count) in Figure 3 and Figures 4A,B. Two-way analysis of variance (ANOVA) was used to compare data meeting the assumption of normal distribution like the path angle adjustment details in Figures 4F,G. Differences were considered statistically significant at *p* < 0.05. GraphPad Prism 8 was used for statistical analysis and graphical plots.

3 Results

3.1 The SMF-induced immediate and reversible inhibition of tadpole swimming

To investigate the effect of the SMF condition on the swimming behavior of the tadpoles, we first used an experimental procedure with different tadpoles for the sham and magnetic treatments (Figures 2A,B). As showing in the swimming distance test, the tadpoles, after sham or magnetic treatment (Figures 2C–F), exhibited typical photo-motor responses with immediate more active movement during light



stimulation than in darkness, and the distance level could recover when the magnet was removed.

For the tadpoles in the magnetic group, the average swimming distance during the 30 s light pulses showed a decreasing trend during the tests compared to those in the

dark adaptation (-21% , $p = 0.2184$); and when the magnet was removed, the swimming distance recovered to the level of the dark adaptation test before treatment ($p = 0.9396$). Although tadpole locomotion also exhibited a magnet-induced decreasing trend during testing in both the dark adaptation period (-13%) and the 30 s dark pulses (-15%) after the light, the magnet-induced changes in swimming distance were not statistically significant compared to either the blank or recovery conditions (Figure 2C).

In the sham treatment, no significant changes were observed in any of the experimental tests, under either bright or dark conditions (Figure 2D). The results indicate that SMF has an inhibitory effect on the locomotion of tadpoles and that the SMF effect is immediate and reversible.

To further confirm the immediate and reversible SMF effect, we analyzed the distance in the last 30 s of the dark adaptation period (270–300 s), the first 30 s of the light stimulation period (300–330 s), and the dark period (330–360 s) in detail (Figures 2E,F). These results were consistent with those obtained from the average swimming distance. During the first 30 s light pulse, the presence of the SMF tended to decrease the swimming distance of the tadpoles (-18%), but this was not statistically significant compared to the blank condition ($p = 0.0778$). However, swimming activity returned to normal when the SMF was removed from the tadpoles ($p = 0.0345$) (Figure 2E). We suggest that the tadpoles may be more sensitive to recovery from the SMF condition than from the weak GMF condition. During the first 30 s dark pulse, tadpoles in the presence of the magnet also exhibited a weak decreasing tendency (-15%) in swimming activity, but no statistical difference from that in the blank and recovery condition, consistent with the analysis using mean swimming distance. The sham treatment showed the same negative effect on swimming distance as shown by the mean distance (Figure 2F).

3.2 SMF exerts locomotion reduction both in dark and bright conditions

To demonstrate SMF-induced inhibition of locomotion, we used the second experimental procedure in the subsequent experiments: for the same batch of tadpoles, each tadpole was tested under the “sham” and “magnet” conditions sequentially (Figure 3B), which provides a better investigation of the SMF effect on the swimming behavior by avoiding possible variations due to differences between individual tadpoles. To compare the effect of SMF in more detail, two additional parameters, active duration and swimming speed, were used in the following experiments.

We observed that SMF inhibited the locomotion of tadpoles in a different pattern under dark and bright conditions. Under the bright conditions, tadpoles maintained a similar active swimming duration, but swimming intensity, *i.e.*, distance

(−31%, $p = 0.0025$) and swimming speed (−32%, $p = 0.0015$), was significantly inhibited by the presence of the magnet (Figure 3C). All three locomotion parameters showed statistical significance decreased during the 30s dark pulse (distance: −26%, $p = 0.0011$; active duration: −26%, $p = 0.0155$; speed: −32%, $p = 0.0007$) (Figure 3D). These results confirm the inhibitory effect of SMF on tadpole locomotion and indicate that the inhibitory pattern of SMF differs between light and dark conditions.

3.3 The SMF weakens the path adjustment behavior and alter the direction preference in dark

The above results showed that the inhibitory effect of the SMF was observed even at low locomotion when the tadpoles rested in the dark, suggesting that the SMF may act on small-scale movement. To test this hypothesis, we examined the effect of the SMF on the fine movement of tadpoles, i.e., their directional adaptation during swimming, using the second experimental procedure (Figures 3A,B).

The total number of path directional adaptations were significantly reduced in both the bright (−34%, $p = 0.0015$) and dark (−55%, $p = 0.0001$) conditions under the SMF (Figures 4A,B), consistent with our hypothesis above, and the reduction are greater than that of the three locomotion parameters analyzed above.

When we break down the distribution of the number of directional adaptations by different angular intervals, we find that tadpoles under bright and dark conditions have different habits adapting the path to the angle. Figures 4C–E shows: 1) more large-angle (45–180°) adjustments in darkness; 2) medium-angle (10–45°) adjustments were preferred by the tadpoles in both the bright and dark conditions, and showed more right preference (+, 23.1%) than left preference (−, 16.7%) in darkness; 3) a right-forward small-angle (0–5°) adjustment preference was shown by the tadpoles in both the bright and dark conditions. In addition, further analysis revealed that, although there was no statistical difference, the tadpoles in the sham group showed a right-sided preference in the medium-angle (10–45°) and small-angle (0–5°) adjustments; whereas in the presence of a magnet, the right-sided preference of tadpoles was reversed to the left-sided preference (−, 21.3% vs + 17.9%). This path angle adjustment preference is not evident in bright conditions (Supplementary Figure S2).

In the presence of a magnet, path angle adjustments of tadpoles showed a general downward trend at all angles under both bright and dark conditions (Figures 4F,G). In the light, the SMF had an obvious inhibitory effect on the left-sided large-angle (45–180°) adjustments; for the dominant medium-angle (10–45°) adjustment, the number of angle adjustments were significantly reduced by the SMF exposure in both the left (−30%, $p = 0.0005$) and right ranges (37%, $p < 0.0001$), indicating no left-right preference. The SMF significantly suppressed the small-angle (0–5°) adjustments (−40%; $p = 0.0046$), which had a

pronounced right-sided preference in the sham group (Figure 4F). In the dark condition, the SMF-exposure significantly reduced the number of large-angle (45–180°) adjustments (left: 57%, $p = 0.0268$; right: 58%, $p = 0.0196$), with no left-right preference, and the SMF-exposure significantly reduced only the number of the right-sided medium-angle (10–45°) adjustments (−67%, $p = 0.0046$) (Figure 4G). The results confirmed that the fine movement of the tadpoles and path angle adjustment behavior during swimming were inhibited by the SMF.

4 Discussion

In this study, we demonstrated an immediate, reversible, and light-independent magnetic response in *Xenopus* tadpoles with mature light-sensing ability (St. 48). The effects were demonstrated in a locomotion test at 30s light plus 30s dark, and the assay was completed with two tests under sham and magnetic conditions. In the presence of the SMF, a sharp decrease in locomotion activity was observed under both dark and light conditions (Figures 2–4), indicating that light is not a critical factor in the tadpoles' behavioral response to an SMF. The SMF-weakened swimming behavior was restored shortly after the magnet was removed. Tadpole swimming distance of the tadpoles returned to the normal levels almost immediately in the subsequent GMF blank recovery test less than 5 min after SMF exposure (Figure 2). The rapid and reversible behavioral SMF response in *Xenopus* would be an ideal behavioral phenomenon for further investigation of the neural and molecular mechanisms of biomagnetic effects.

Recent studies have provided evidence supporting the existence of a light-independent magnetic response mechanism in animals. For example, *Fukomys mechowii* and *Heliophobius argenteocinereus* can obtain directional information from a magnetic field even in the dark [42]. Myklatun *et al.* also pointed out that adult fish can orient themselves according to the direction of the magnetic field even in a dark and unfamiliar environment [8]. Our results showed that SMF-induced reductions in key locomotion indexes (distance speed, active duration, and number of path angle adjustments) were recorded even during low-intensity swimming of tadpoles in darkness, and these changes were statistically significant. Overall, by examining the SMF effect on fine movement in detail, we confirmed the inhibitory effect of the SMF in the tadpole under dark conditions, which is consistent with our hypothesis that light-independent SMF-sensation exists in the tadpole. In addition, it has been reported that GMF-perceptive bats rely more heavily on information from light for navigation under bright conditions, such as using sunsets to calibrate a magnetic compass [43]. In other words, under light conditions, animals are less dependent on the magnetic field than on information provided by light, whereas in the dark, when there is no light information, animals are more sensitive to changes in the magnetic field, which is consistent with our experimental results. In commonly used animal models, tadpoles are cheap, easy to obtain, and amenable to genetic

manipulation. Therefore, our experimental paradigm will help overcome the lack of animal models suitable for studying the mechanism of instantaneous response to the magnetic field.

Although we observed that light is not a necessary factor for the SMF-effect in the tadpoles, we also found that the SMF-response pattern in the light was different from that in the dark, showing no left-right preference, and was easily detected with a regular parameter (swimming distance). Since the photosensing system would trigger both locomotion and magnetoresponse, light could be a possible factor whose effects mask those of the magnet on directional preference in the light. As the locomotion activity of the tadpoles was much more elevated by light stimulation, it was not easy to detect whether the SMF effect was left-right biased in the light conditions. Interestingly, recent studies have indicated that the strength of the magnetic field has a more important effect on bird navigation than the inclination [44]. Therefore, it will be interesting to explore how the magnetic field strength is perceived in the dark using this model, which will help to unravel the mystery of magnetoreception.

Many reports have shown that most bio-magnetic responses of animals are weak but accumulative, so that a long-term magnetic field exposure would promise the accumulation of sufficient modifications at transcription, expression, or signal transduction levels leading to a detectable bio-magnetic response [45–48]. However, the immediate and reversible response of tadpoles to SMF exposure observed in our experiments suggests that a rapid biological process serves as the basis for the mechanism rather than the time-consuming processes mentioned above. Magnetic fields have been shown to affect energy metabolism. For example, magnetic field exposure can cause changes in ATP content, decreased mitochondrial activity, and changes in the mitochondrial membrane potential [2, 49–53]. Exercise is an energy-consuming process and energy metabolism is a fast and efficient process. Therefore, it is reasonable to study the mechanisms from the perspective of energy metabolism. In addition, magnetic fields have been reported to be related to neural conduction, for example, by affecting the activity of neurons or ion channels [54–57]. Therefore, nerve conduction is a potential target for magnetic fields. Moreover, based on the current experimental evidence, we could not exclude the possibility that CRYs are involved in the SMF-inhibitory effect. It has been shown that type 2 CRYs could also function as magnetosensors even in the dark [58, 59]. It is hypothesized that CRY undergoes a magnetically sensitive radical-pair step in the light, and there are a large number of activated CRY molecules, so magnetic sensitivity in the dark is possible [58, 60]. In the future, it is hoped that this model can be combined with real-time electrical activity detection and other technologies to determine the neural circuitry of biological magnetoreception.

In summary, we have demonstrated a rapid and light-independent SMF response behavior in tadpoles. The SMF significantly reduces the locomotion intensity, and the

tadpoles can perceive changes of the magnetic field, even in the dark. In addition, the SMF was able to alter the tadpoles' movement angle adjustment preference pattern in the dark. Our experimental paradigm provides novel behavioral evidence for the intrinsic light-independent magnetic response and clues to uncover the mechanism of the magnetoresponse with a practical system *in vivo* for further investigation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by institutional ethical guidelines (grant No. SYXK2014-30).

Author contributions

YL, RH, and TJ contributed to the study design. WM, HZ, and XW performed the experiments. WM, JR, YL analyzed the data. WM, JR, YL and RH wrote the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphy.2022.995860/full#supplementary-material>

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