Lizards respond to an extremely low-frequency electromagnetic field

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SUMMARY

Animals from a wide range of taxa have been shown to possess magnetic sense and use magnetic compasses to orient; however, there is no information in the literature on whether lizards have either of these abilities. In this study, we investigated the behavioral responses of a diurnal agamid lizard (*Pogona vitticeps*) to a sinusoidal extremely low-frequency electromagnetic field (ELF-EMF; 6 and 8 Hz, peak magnetic field 2.6 μT, peak electric field 10 V m^{-1}). Fourteen adult lizards were divided randomly into two groups (the EMF and control groups; each group had three males and four females). The EMF group received whole-body exposure to ELF-EMF and the control group did not. Lizards in the EMF group were exposed to ELF-EMF for 12 h per day (during the light period). The number of tail lifts was monitored beginning 3 days before exposure and ending after 5 days of exposure. For each individual, the average number of tail lifts per day was calculated. The average number of tail lifts per individual per day was greater in the EMF group than in the control group (20.7±6.3 and 9.1±4.5 tail lifts, respectively, *N*=7 each, *P*=0.02). We confirmed the reproducibility of this response by a cross-over trial. These results suggest that at least some lizards are able to perceive ELF-EMFs. Furthermore, when the parietal eye of the lizards was covered with a small round aluminum 'cap' which could block light, the tail-lifting response to ELF-EMF disappeared. Our experiments suggest that (1) lizards perceive EMFs and (2) the parietal eye may be involved in light-dependent magnetoreceptive responses.

Key words: ELF-EMF, lizard, magnetic sense, magnetoreception, tail behavior, tail lifting.

INTRODUCTION

In their review of magnetoreception in animals, Wiltschko and Wiltschko (Wiltschko and Wiltschko, 2006) present evidence showing that animals from a wide range of taxa possess magnetic sense and use magnetic compasses to orient. Such taxa include mollusks, crustaceans, insects, fishes, birds, mammals and amphibians (Wiltschko and Wiltschko, 2006). Among reptiles, loggerhead sea turtles are thought to migrate using the geomagnetic field (Light et al., 1993). Non-migratory animals such as mice (Mather and Baker, 1981) and rats (Burda et al., 1990) also reportedly have magnetic sense, but studying sensitivity to electromagnetic field (EMF) in such species is difficult because the approaches used – such as attempting to train them to exhibit a magnetic orientation – are extremely time-consuming and often unsuccessful because of the difficulty in motivating the animals, etc. Because a variety of animals are reported to show changes in behavior in advance of major earthquakes (Kirschvink, 2000; Li et al., 2009; Yokoi et al., 2003), high sensitivity to ultra low-frequency (ULF) or extremely low-frequency (ELF)-EMF signals that often precede major earthquakes has been suggested as a possible basis for these responses. The thresholds of sensitivity to changes in the geomagnetic field are reported to be in the range of 10–200 nT (Walker et al., 2002) and magnetoreception mechanisms involving magnetite are sensitive to ELF-EMFs up to ≈10 Hz (Kirschvink, 2000). ULF- and ELF-EMF signals that precede large earthquakes may be as high as 0.1 nT to a few tens of nanotesla within a 60–100 km radius of the epicenter (Hayakawa et al., 2007; Kirschvink, 2000). Behavioral changes in response to ELF-EMFs

provide a novel approach for studying sensitivity to EMFs in animals that are not well suited for use in assays involving (migratory or non-migratory) orientation behavior. To the best of our knowledge, no non-migratory reptiles have yet been found to have magnetic sense. In the present study we selected the non-migratory diurnal agamid lizard *Pogona vitticeps* (De Vosjoli et al., 2001) with the aim of determining whether this reptile has magnetic sense. We exposed *P. vitticeps* to ELF-EMFs and observed them to determine whether this non-migratory lizard species is behaviorally sensitive to ELF-EMFs.

Because experimental evidence is accumulating that the threshold sensitivity of some animals to magnetic fields is less than a few tens of nanotesla (Walker et al., 2002), as a first step we exposed lizards to ELF-EMFs in the order of microtesla. In a pilot study, in which we evaluated the effects of an ELF-EMF on *P. vitticeps,* a continuous ELF-EMF for a few days evoked tail-lifting in the lizards more frequently during daytime hours (T.N., unpublished data). In addition, we observed more locomotion in the lizards when a combination of 6 and 8 Hz ELF-EMFs were used (a pair of coils, one emitting 6 Hz, the other emitting 8 Hz) than when either frequency was used alone (T.N., unpublished data). Tail-lifting behavior in lizards, where the tail is raised for a period of a few seconds to a few minutes, is thought by some to be an anti-predator defensive posture that increases the potential for survival by preventing predators from attacking the lizard's head (Sherbrooke and Middendorf, 2004). The lizard *Leiocephalus carinatus* curls its tail as a component of display during intraspecific agonistic encounters and courtship (Cooper, 2001). Furthermore, it has been

reported that the pineal organ of salamanders may be capable of light-dependent magnetoreception (Deutschlander et al., 1999a; Deutschlander et al., 1999b).

In the present study, we attempted to examine the behavioral responses of *P. vitticeps* to an EMF and also to study whether or not the parietal eye of *P. vitticeps* is involved in a magnetoreceptive reaction by monitoring these behavioral responses.

MATERIALS AND METHODS

Adult central bearded dragons (*Pogona vitticeps* Ahl; Agamidae) were obtained from a commercial source (Daiwa Pet Co., Kyoto, Japan) and we bred juveniles ourselves. Individuals were easily recognizable on the basis of their morphological features. Different lizards were used in experiments 1 and 2. Four adult lizards used in experiments 2 were also used in experiment 3. All animal experiments were approved by the Kyoto University Animal Research Committee.

Experiment 1

Six adult males and eight females [mean body mass (M_b), 278.1±73.3 g; mean snout–vent length (*SVL*), 17.5±1.9 cm; mean total length (*TL*), 41.3±5.2 cm] were used in experiment 1. The lizards were divided randomly into two groups of seven lizards each (the EMF and control groups), such that each group had three males and four females. We used two terrariums (60 cm×45 cm×45 cm; length × width × height) and each terrarium was divided in half (30 cm×45 cm×45 cm) with a wooden board (Fig. 1A). Each lizard was kept in a separate half of a terrarium, which had paper sheets fixed to the sides to prevent any visual contact between individuals. That is, pairs of lizards in the same experimental treatment group were housed in the same terrarium, with the wooden separator to keep them apart. We compared two lizards in the EMF group and two lizards in the control group at the same time and repeated the experiment four times. The two terrariums were kept in the same room at 27.0±1.0°C and a relative humidity of 50±5%. The terrariums were subject to a 12 h:12 h light:dark cycle, with white and ultraviolet light (≈1500 lux; lights on at 09:00 h). All lizards had access to a standard diet and tap water *ad libitum*. The minimum distance between the EMF and control groups was 2.5 m.

The number of tail lifts performed by each lizard was monitored beginning 3 days before exposure to an EMF and ending after 5 days of exposure. For each individual, the average number of tail lifts per day was calculated. Baseline values were determined using measurements taken in the 3 days before exposure. The ratio of values taken on each day during exposure or control exposure to the baseline value was then calculated. Values were compared for the two experimental groups for each day of the 5-day exposure period.

We ensured that the EMF and control group terrariums were approached by people only when necessary for feeding, and that the terrariums were approached an equal number of times with the minimum possible disturbance.

The EMF group received whole-body exposure to an ELF-EMF for 12 h day^{-1} (9:00–21:00 h) for 5 days. The EMF group was subjected to ELF-EMF during the light period only, because in a preliminary study we found that lizards subjected to continuous EMF exposure displayed tail-lifting behavior mainly during daylight hours. The control group was not experimentally exposed to any EMF except for civil EMF noise (a few nanotesla).

The ELF-EMF was generated by an electromagnetic device (Ichikawa Construction Co., Gifu, Japan) consisting of one pair of square-shaped coils mounted in two frames (100 cm× 100 cm×2.5 cm; length × height × diameter; Fig. 1A). There were
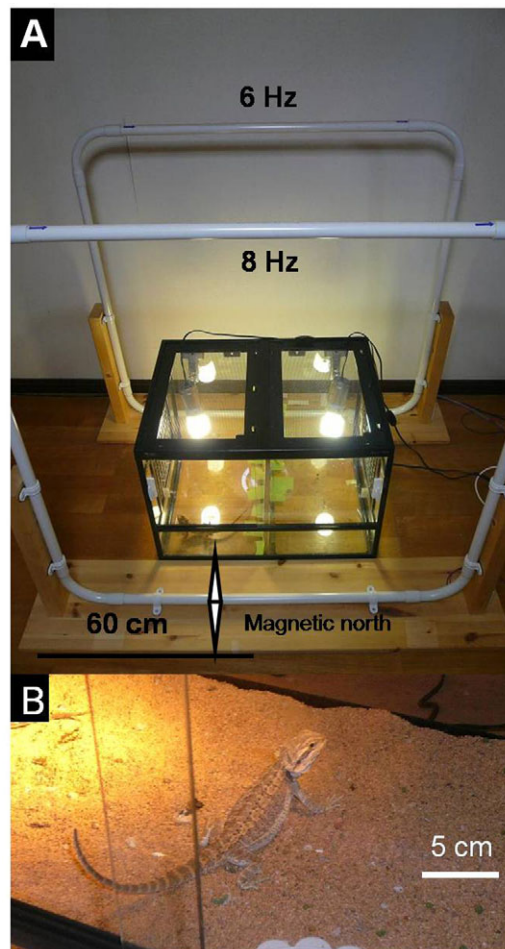

Fig. 1. (A) The ELF-EMF exposure device composed of one pair of square coils installed in two vinyl plastic frames. (B) A *P. vitticeps* lizard displaying a tail-lifting posture.

100 turns in each coil. The frames of the coils were made of vinyl plastic (Mirai Industry Co. Ltd, Gifu, Japan). Using a vibrometer (Model-1332A, Showa Sokki, Tokyo, Japan) we determined that there was no detectable vibration in these coils. The axes of the coils were parallel to the geomagnetic field. The terrarium was placed between the two coils (Fig. 1A). The distance between the coil frames was 70 cm and the coil frames were placed at a distance of 12.5 cm from the sides of the terrarium. The paired coils produced a sinusoidal 6 and 8 Hz EMF with a peak magnetic field of 2.6 µT and a peak electric field of 10 V m^{-1}. The device produced no audible noise. The EMF was controlled by a functional generator (FG-274; TEXIO Corporation, Tokyo, Japan), and peak values were measured using an EMF meter (ME3830B; Gigahertz Solutions GmbH, Langenzenn, Germany). There were no metal items except an ultraviolet light source inside the exposure system that could have interfered with the magnetic field.

To monitor the number of tail lifts, images of each terrarium were captured automatically every minute for 24 h day^{-1} during the experimental period using two web cameras (CG-NCMNV2; Corega K.K., Kanagawa, Japan) placed in front of the terrariums and connected to a PC. In addition, to monitor the duration of each tail lift, moving images of each terrarium were record on video tapes for 3 h day^{-1} (9:00–12:00 h) during the experimental period using two video cameras (DCR-PC350; Sony Corporation, Tokyo, Japan)

placed in front of the terrariums. We defined tail lifting as a posture in which the lizard's tail formed an angle of more than 30 deg with the ground (Fig. 1B). In this study, two people measured the number of tail lifts independently. One of them knew whether an experimental or control sample was being handled, and the other person did not. We compared and discussed each set of results and decided when they were different. If the body axis of a lizard was at a right angle to the camera, we measured a 30 deg tail angle using a protractor. In addition, we calculated the average tail apex height from the bottom of the terrarium for each lizard. If the body axis of a lizard was not at right angles to the camera, we defined a tail lift as being over 30 deg when tail apical height exceeded the average value.

While the lizards were exposed to ELF-EMFs, their tail and body movements were observed more frequently than during the pre-exposure period. Tail lifts by lizards that were not moving were thought not to be important. In addition, it was possible that tail lifts while stationary could be due to the animal leaning against the side of the cage. Therefore, we defined a cut-off value as four times during 4 min for stationary tail lifts to calculate number of tail lifts. Most tail lifts lasted less than 4 min; indeed, during our observations (video data) only 1.7% (8/464) of the tail lifts were longer than 4 min (their lifted tails rarely maintained the same position for more than 4 min).

For each individual, the following were calculated: (1) the average number of tail lifts per day, (2) the average ratio of the number of tail lifts on exposure days to the baseline value, and (3) the average duration of each tail lift. Experiment 1 was carried out in summer (June 15–August 3, 2008).

Experiment 2

We also conducted a cross-over trial using four adult male and four adult female lizards (mean M_b, 346.1±56.9 g; mean SVL, 19.3±1.2 cm; mean TL, 42.7±7.4 cm) to confirm the repeatability of the results found in experiment 1. The lizards were divided randomly into two groups of four (EMF and control group), such that each group had two males and two females. In brief, the experiment was run twice, with the lizards that were in the control group first going into the EMF group the second time around, and *vice versa*. This means that each lizard underwent both EMF and control treatments. They were given a 3-day rest period between treatments. The experimental set-up was the same as for experiment 1, with two terrariums divided in half using a wooden board, and the lizards kept in a separate half terrarium with sheets of paper fixed to the sides to prevent visual contact. We compared two lizards in each of the EMF and control groups at the same time and repeated the experiment twice. The minimum distance between the individuals in the EMF and control groups was 2.5 m.

As with experiment 1, the number of tail lifts was monitored, beginning 3 days before exposure to an EMF and ending after 5 days of exposure. For each individual, the average number of tail lifts per day was calculated. Baseline values were determined using measurements taken over the 3 days before exposure. The ratio of values taken on each day during exposure or control exposure to the baseline value was then calculated. Values were compared for the two groups for each day of the 5-day exposure period. Experiment 2 was carried out in winter (November 4–December 30, 2008). Other methods were the same as those described for experiment 1.

Experiment 3

We examined whether or not the parietal eye of *P. vitticeps* (Fig. 2A) is involved in the magnetoreceptive response by monitoring the behavioral responses of the lizards to an EMF. We used four adult and eight juvenile lizards. Eight males and four females (mean
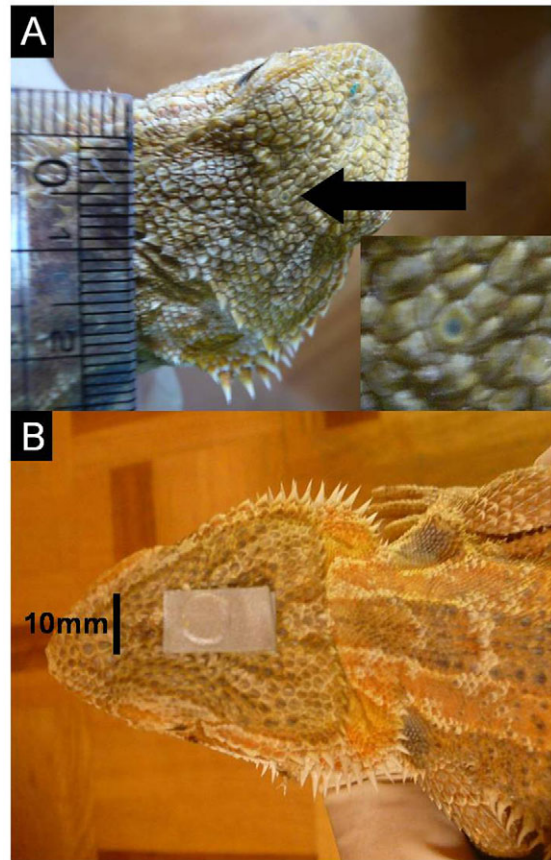

Fig. 2. (A) The parietal eye of *P. vitticeps* (arrow). (B) Small round aluminum 'cap' (5 mm in diameter, 0.2 mm thick, and 0.01 g mass) covering the parietal eye of *P. vitticeps*.

M_b, 167.0±164.9 g; mean SVL, 13.4±5.1 cm; mean TL, 29.8±13.3 cm) were used in experiment 3. The lizards were divided randomly into two groups of six lizards each (the EMF and control groups), such that each group had four males and two females. In experiment 3, we added one more experimental set-up which consisted of four terrariums (30 cm×45 cm×30 cm; length × width × height) and coils to use for four smaller juvenile lizards. Each lizard was kept in a terrarium, which had paper sheets fixed to the sides to prevent any visual contact between individuals. We used the same experimental set-up as in experiment 2 for four adult and four larger juvenile lizards. In brief, the experiment was run twice, with the lizards that were in the control group first going into the EMF group the second time around, and *vice versa*. This means that each lizard underwent both EMF and control treatments. Then, we compared 12 lizards in the EMF group with 12 lizards in the control group. In both groups, small round aluminum 'caps' (5 mm in diameter, 0.2 mm thick, and 0.01 g mass; Hikari Co., Osaka, Japan) were attached to the dorsal surface of the head of each lizard using tape (PH-1813, DKH, Tokyo, Japan; Fig. 2B) and remained in place during the baseline period (3 days) and the exposure period (5 days). That is, the parietal eye of all lizards was covered with an aluminum cap during the experiment. We did not measure the duration of each tail lift because this can be quite variable. The experiment was carried out at the end of winter/beginning of spring (February 5–March 17, 2009). Other methods were the same as those described for experiment 2.

Statistical analysis

Statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA). All values are expressed as means ± s.d. $P<0.05$ was considered to be statistically significant.

RESULTS
Experiment 1

The average number of tail lifts per individual per day was greater in the EMF group than in the control group (20.7±6.3 tail lifts in the EMF group vs 9.1±4.5 tail lifts in the control group, $N=7$ each, $P=0.02$; Wilcoxon rank-sum test; Table 1). The average ratio of the number of tail lifts on exposure days to the baseline value was also significantly greater in the EMF group than in the control group (2.0±0.6 in the EMF group vs 0.8±0.4 in the control group, $N=7$ each, $P=0.02$; Wilcoxon rank-sum test; Table 1).

The average duration of tail lifts per individual per day was not statistically significantly longer in the EMF group than in the control group (32.2±55.7 s in the EMF group vs 18.8±35.3 s in the control group, $N=7$ each, $P=0.09$; Wilcoxon rank-sum test).

Experiment 2

Two data sets – from lizards exposed to EMF first and control conditions second, and from those exposed to control conditions first and EMF second – were combined and analyzed. The average number of tail lifts per individual per day was greater in the EMF group than in the control group (7.9±8.7 tail lifts in the EMF group vs 1.8±3.2 tail lifts in the control group, $N=8$ each, $P<0.001$; Wilcoxon signed-rank test; Table 2). The average ratio of the number of tail lifts on exposure days to the baseline value was also significantly greater in the EMF group than in the control group (2.0±0.5 in the EMF group vs 0.7±0.4 in the control group, $N=8$ each, $P<0.001$; Wilcoxon signed-rank test; Table 2). The average duration of tail lifts per individual per day did not differ between the two groups (33.0±62.0 s in the EMF group vs 13.2±22.8 s in the control group, $N=8$ each, $P=0.87$; Wilcoxon rank-sum test).

Experiment 3

In this cross-over study, two types of data – from lizards exposed to EMF first and control conditions second, and from those exposed to control conditions first and EMF second – were combined and analyzed. For all lizards, in the exposure period the average number of tail lifts per individual per day was not significantly different between groups (24.2±32.1 tail lifts in the EMF group vs 20.4±23.5 tail lifts in the control group, $N=12$ each, $P=0.57$; Wilcoxon signed-rank test; Table 3). In the exposure period, the average ratio of the number of tail lifts on exposure days to the baseline value was not significantly different between the groups (1.1±0.5 in the EMF group vs 0.9±0.3 in the control group, $N=12$ each, $P=0.50$; Wilcoxon signed-rank test; Table 3). We present separate values for tail lifting in juveniles and adults for both the baseline and experimental treatment conditions (Table 3). For adults and juveniles, in the exposure period, the average number of tail lifts per individual per day was not significantly different between groups (adults:

11.6±13.6 tail lifts in the EMF group vs 9.6±13.5 tail lifts in the control group, $N=4$ each, $P=0.42$; juveniles: 30.5±36.7 tail lifts in the EMF group vs 25.8±25.6 tail lifts in the control group, $N=8$ each, $P=0.76$; Wilcoxon signed-rank tests; Table 3). For adults and juveniles, in the exposure period, the average ratio of the number of tail lifts on exposure days to the baseline value was not significantly different between the groups (adults: 1.1±0.5 in the EMF group vs 0.9±0.6 in the control group, $N=4$ each, $P=0.50$; juveniles: 1.1±0.5 in the EMF group vs 0.9±0.2 in the control group, $N=8$ each, $P=0.50$; Wilcoxon signed-rank tests; Table 3).

DISCUSSION

The average ratios for number of tail lifts on an EMF exposure day relative to baseline levels were very similar in experiments 1 and 2 (2.0±0.6 in experiment 1 and 2.0±0.5 in experiment 2; Tables 1 and 2). This confirms the reproducibility of experiment 1 based on the results of experiment 2. However, the average number of tail lifts per day differed in experiments 1 and 2, which is very likely to be due to seasonal factors because experiment 1 was conducted in summer and experiment 2 in winter. In winter, once mature (after 1 year of age), $P. vitticeps$ usually enter a state of brumation (reptilian winter sleep), commonly termed shutdown, in which they remain relatively inactive, hidden in shelters or lying on the ground and eating little if at all (De Vosjoli et al., 2001). Only in experiment 3 did we use eight juvenile lizards, which ate more food and showed more activity and a greater number of tail lifts than adult lizards.

Table 1. Results from experiment 1: average number of tail lifts and ratio of the number of tail lifts on an exposure day relative to baseline levels

	Control group		EMF group	
	No. of tail lifts	Ratio[†]	No. of tail lifts	Ratio[†]
Pre-exposure baseline	11.5±10.8	1	10.3±17.2	1
Exposure day				
Day 1	6.4±8.1	0.6	25.9±28.3	2.5
Day 2	9.1±7.9	0.8	10.1±10.8	1.0
Day 3	16.9±18.1	1.5	22.1±45.1	2.2
Day 4	7.0±6.3	0.6	20.3±29.9	2.0
Day 5	6.1±5.7	0.5	25.2±50.9	2.4
Days 1–5 combined	9.1±4.5	0.8±0.4	20.7±6.3	2.0±0.6

[†]The ratio is the number of tail lifts on a given exposure day or control exposure day divided by the baseline number of tail lifts.

Values are means ± s.d. per individual per day; $N=7$ for each group.

Table 2. Results from experiment 2, a cross-over study: average number of tail lifts and ratio of the number of tail lifts on an exposure day relative to baseline levels

	Control group*		EMF group*	
	No. of tail lifts	Ratio[†]	No. of tail lifts	Ratio[†]
Pre-exposure baseline	2.8±4.4	1	3.9±4.1	1
Exposure day				
Day 1	2.4±2.1	0.9	9.5±13.1	2.4
Day 2	0.0±0.0	0.0	4.0±6.4	1.0
Day 3	3.4±4.9	1.2	9.3±8.4	2.4
Day 4	1.3±3.5	0.4	7.6±9.5	1.9
Day 5	2.1±2.9	0.8	9.1±4.9	2.3
Days 1–5 combined	1.8±3.2	0.7±0.4	7.9±8.7	2.0±0.5

*Two types of data (EMF followed by control treatment and control followed by EMF treatment) were combined and analyzed. See the Materials and methods section for further details.

[†]The ratio is the number of tail lifts on a given exposure day or control exposure day divided by the baseline number of tail lifts.

Values are means ± s.d. per individual per day; $N=8$ for each group.

These differences would explain why the baseline average number of tail lifts per day was greater in experiment 3 than in experiments 1 and 2. In fact, in the data from the four adults used in experiment 3, baseline values for the average number of tail lifts per individual per day were 11.0±10.7 tail lifts in the EMF group and 10.5±10.3 tail lifts in the control group (Table 3) and these baseline values correspond with those of experiment 1.

Our results suggest that lizards can perceive an ELF-EMF (6 and 8 Hz, peak 2.6 μT, 10 V m^{-1}). Two hypotheses for magnetoreception have been discussed in the literature: one proposes a chemical compass based on a radical pair mechanism and the other postulates processes involving magnetite particles (Wiltschko and Wiltschko, 2005). Magnetite-based mechanisms which use the geomagnetic field for 'map' information would require that an animal be sensitive to very small changes in the inclination, or intensity, of the magnetic field in order to sense the spatial variation over their home range or migratory route (Deutschlander et al., 1999b). Experimental evidence is accumulating that the threshold sensitivity of some animals to magnetic fields is less than a few tens of nanotesla, a shift of no more than a few parts in 10^4 in the background field of the Earth (approximately 50 μT) (Walker et al., 2002). Threshold sensitivities of about 25 nT and 200 nT, respectively, have been measured behaviorally in honeybees and electrophysiologically in the bobolink, a bird that migrates between the northern and southern hemispheres (Walker et al., 2002). Estimated sensitivities on the basis of field studies range between 10 nT and about 50 nT in homing pigeons, sharks and whales (Walker et al., 2002). By contrast, since a radical pair mechanism is likely to be insensitive to small changes in magnetic field parameters, a radical pair magnetoreceptor could not be used to 'map' information from the magnetic field (Ritz et al., 2004; Rodgers and Hore, 2009).

In experiment 2, a carry-over effect was not shown (N=8 each, P=0.51; analysis of variance). That is, the difference of exposure timing, with either EMF treatment first and control treatment second, or vice versa, did not affect the number of tail lifts.

In general, tail displays directed by lizards to predators at close range deflect attacks to the tail (Cooper, 2001). In particular, some lizards often autotomize their own tails to escape from predators (Cooper, 2001). Tail displays also appear to be pursuit-deterrent signals in two non-agamid lizard species, Cophosaurus texanus and Callisaurus draconoides (Cooper, 2001). Pursuit-deterrent signaling behavior might visually inform predators of their detection by the prey and eventually reduce the hunting success of predators (Cooper, 2001). The lizard Leiocephalus carinatus curls its tail as a component of display during intraspecific agonistic encounters and courtship (Cooper, 2001).

Terrestrial nocturnal rodents have been reported to perceive low-intensity electric fields; for example, as low as 1.8 mV m^{-1} in rats (Smith et al., 1994) and 6 mV m^{-1} in mice (Smith and Justesen, 1977). Therefore, it is certainly possible that the electric-field intensity used in the present study (10 V m^{-1}) would be strong enough to induce behavioral effects in a wide variety of animals. In lizards, however, which sensory systems are involved (i.e. whether a magnetic or electric sensor, or both sensors) remains unknown.

In experiment 3, in the exposure or control exposure period, when the lizards were wearing aluminum caps, the average number of tail lifts per individual per day did not differ significantly between groups. In order to examine whether irritation caused by attaching the aluminum caps themselves may have affected tail-lifting behavior, we carried out another experiment. We used four adult male and four juvenile female lizards that had been used in experiment 3. The number of tail lifts performed by each lizard was monitored during the baseline non-cap period (3 days) and cap period (3 days). During the cap period, the aluminum caps were attached to the dorsal surface of the head of each lizard using tape. Baseline values for the average number of tail lifts per individual per day were 21.4±19.6 tail lifts during the non-cap period and 19.1±27.3 tail lifts during the cap period (N=8, P=0.74, t-test). Therefore, caps on the lizards' heads did not significantly affect the number of tail lifts. These results strongly suggest that the parietal eye of lizards contributes to light-dependent magnetoreception, as shown previously for salamanders (Deutschlander et al., 1999a; Deutschlander et al., 1999b). There have been no reports on the parietal eye of P. vitticeps, but there are many reports regarding the parietal eyes of other lizards. The parietal eye is present in some species of lizards (Squamata) and in the tuatara (Rhynchocephalia) and both the pineal gland and the parietal eye are

Table 3. Results from experiment 3, a cross-over study: control group with caps *versus* EMF group with caps: average number of tail lifts and ratio of the number of tail lifts on an exposure day relative to baseline levels

	Control group*		EMF group*	
	No. of tail lifts	Ratio[†]	No. of tail lifts	Ratio[†]
All lizards				
Pre-exposure baseline	23.7± 27.7	1	22.8±21.9	1
Exposure day				
Day 1	14.9±12.8	0.6	25.2±28.1	1.1
Day 2	18.8±15.0	0.8	15.3±18.7	0.7
Day 3	15.6±19.8	0.7	37.5±48.4	1.6
Day 4	30.6±40.1	1.3	11.5±11.6	0.5
Day 5	22.0±19.6	0.9	31.7±37.5	1.4
Days 1–5 combined	20.4±23.5	0.9±0.3	24.2±32.1	1.1±0.5
Adult lizards				
Pre-exposure baseline	10.5±10.3	1	11.0±10.7	1
Exposure day				
Day 1	7.0±4.8	0.7	8.5±8.7	0.8
Day 2	5.5±3.7	0.5	9.3±9.9	0.8
Day 3	3.8±6.2	0.4	16.0±17.3	1.5
Day 4	17.8±28.3	1.7	5.5±3.7	0.5
Day 5	13.8±9.5	1.3	18.8±23.0	1.7
Days 1–5 combined	9.6±13.5	0.9±0.6	11.6±13.6	1.1±0.5
Juvenile lizards				
Pre-exposure baseline	30.3±31.4	1	28.7±23.8	1
Exposure day				
Day 1	18.9±14.0	0.6	33.5±31.2	1.2
Day 2	25.5±13.9	0.8	18.3±21.8	0.6
Day 3	21.5±22.0	0.7	48.3±56.1	1.7
Day 4	37.0±45.2	1.2	14.5±13.2	0.5
Day 5	26.1±22.6	0.9	38.1±42.9	1.3
Days 1–5 combined	25.8±25.6	0.9±0.2	30.5±36.7	1.1±0.5

*Two types of data (EMF followed by control treatment and control followed by EMF treatment) were combined and analyzed. See the Materials and methods section for further details.

[†]The ratio is the number of tail lifts on a given exposure day or control exposure day divided by the baseline number of tail lifts.

Values are means ± s.d. per individual per day; N=4–12 for each group.

photosensitive (Tosini, 1997). The parietal eye may develop as an outgrowth of the pineal gland. In particular, the parietal eye is a highly organized photoreceptive structure, with a well-defined lens, cornea and retina and the most important (and studied) secretory product of this complex is the hormone melatonin which is synthesized by both organs (pineal gland and parietal eye) (Tosini, 1997). The pineal organ is believed to be the neuroendocrine transducer of changes in photoperiod and environmental temperature and it has been demonstrated to have a functional role in many aspects of reptilian biology (Tosini, 1997).

In salamanders, photoreceptors were found to be located in the pineal organ, the ancient third eye of vertebrates, which in amphibians is directly sensitive to light (Adler, 1976). Critical tests, in which the skull above the pineal organ was covered with a colored filter, but where the eyes were open to natural light, clearly showed that the magnetic compass in salamanders depends solely on the spectral properties of the light reaching the pineal organ (Deutschlander et al., 1999a; Deutschlander et al., 1999b). Salamanders with clear caps orientated perpendicular to the shoreward magnetic axis under long-wavelength light, indicating that the caps did not alter the orientation response (Deutschlander et al., 1999a). By contrast, salamanders with caps that only allowed long wavelengths to pass through, thus blocking short wavelengths, exhibited bimodal magnetic orientation parallel to the shoreward axis in training tanks (Deutschlander et al., 1999a). Covering the dorsal surface of the salamander's head with a short-wavelength-blocking filter therefore mimicked the effect of long-wavelength training on the salamander's shoreward compass response (Deutschlander et al., 1999a). Thus, extraocular photoreceptors are involved in the light-dependent magnetic compass of salamanders (Deutschlander et al., 1999a).

Studying sensitivity to EMFs in a non-migratory species is difficult because the approaches that have been used in studies of such species – such as attempting to train them to exhibit a magnetic orientation – are extremely time-consuming and often unsuccessful because of the difficulty of motivating the animals, etc. In addition to training the animals, special equipment is needed to conduct such experiments (e.g. Kirschvink et al., 1997). By using tail-lifting behavior in lizards as a magnetoreceptive reaction, our experiments have a number of advantages: (1) our experimental animals do not need training and habituating, (2) our experiments do not require specialized devices with which to conduct training and habituating, (3) *P. vitticeps* are easily available because they are one of the most popular species of pet lizards, are relatively hardy and easy to keep compared with many other reptiles, and are naturally tame, and (4) the parietal eye can easily be shaded. For these reasons our new method will be useful in future research into the magnetoreceptive ability of animals.

With regard to light-dependent magnetoreception and the involvement of the parietal eye in the light-dependent magnetoreceptive response, we could not conduct an experiment in total darkness using an infrared camera because these lizards are diurnal and do not act under total darkness. Transparent caps may also serve as a useful control for stress to reconfirm the effect during

daytime hours. Kirschvink (Kirschvink, 2000) proposed that a magnetite-based magnetoreception mechanism would be capable of detecting the magnetic anomalies associated with earthquakes. However, there have been no reports of magnetite in the pineal complex of reptiles or amphibians (i.e. in the pineal organ, frontal organ or parietal eye); nor has it been suggested that this is a probable site for a magnetite-based receptor. The relationship between sensitivity to external electric fields and the theoretical lower limit of neural-network sensitivity is unclear, and the precise mechanisms remain to be elucidated.

In summary, our experiments suggest that (1) lizards perceive EMFs and (2) the parietal eye may be involved in the light-dependent magnetoreceptive response. Further investigation will be necessary to gain a better understanding of how lizards respond to EMFs, the mechanism behind EMF perception, and its adaptive significance.

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