

INTERACTIONS OF LIGHT AND GRAVITY RECEPTION WITH MAGNETIC FIELDS IN *XENOPUS LAEVIS*

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Summary

Tadpoles of *Xenopus laevis* are shown to respond to magnetic fields using a gravity-related test paradigm. They exhibit a directional selectivity with respect to angular variations of the inclination of low-intensity magnetic fields. This response is abolished when the optic nerves of the tadpole are transected, indicating that the peripheral optical system is involved in the detection of magnetic fields.

Introduction

The ability to perceive the Earth's magnetic field has now been demonstrated in a variety of animals. The spectrum of species sensitive to magnetic fields (MFs) ranges from prokaryotic organisms (Delgado, 1985) to molluscs (Kavaliers and Ossenkopp, 1988), crustaceans (Pardi *et al.* 1985), arthropods (Martin and Lindauer, 1977) and chordates, including man (Kalmijn, 1978; Semm *et al.* 1980; Cremer-Bartels, 1984).

Despite the growing evidence for MF perception in animals the question of how and where MFs are detected by the organism remains obscure. On the basis of theoretical considerations, a connection between light reception and MF reception has repeatedly been postulated (Leask, 1977; Schulten and Windemuth, 1986). Experimental evidence for this idea has been demonstrated in several cases (Cremer-Bartels, 1984; Leucht, 1984; Semm and Demaine, 1986). The receptive structures responsible for the detection of gravity have been suggested as another site of MF action. The now classical experiments of Martin and Lindauer (1977) have shown a relationship between gravity detection and MF detection by demonstrating that the direction indicated by dancing honeybees is influenced by the daily variations in intensity of the Earth's magnetic field. The combination between MF and gravitational stimulus processing has been shown not only at the behavioural level but also by using electrophysiological techniques (Semm *et al.* 1984; Korall and Martin, 1987).

Larvae of the South African clawed toad *Xenopus laevis* are midwater, microphagous suspension feeders and exhibit a characteristic angle of body axis

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orientation with respect to gravity. Using this orientational behaviour, two problems are considered. The first is the possible influence of varying MF stimuli on gravity perception in *X. laevis* (i.e. the question of whether body axis orientation is modulated in these animals when the angle of inclination of the MF is changed); the second is to discover if the peripheral optical system is involved in the reception and processing of MF stimuli.

Materials and methods

Tadpoles of *X. laevis* at stage 51–54 were used (Normal Table of Nieuwkoop and Faber, 1956). Groups of 15 animals were placed in a narrow, water-filled glass cuvette (height 20 cm; width 20 cm; depth 1 cm). The animals were free to move inside the container. The cuvette was adjusted in the N–S direction and located in the centre of two pairs of Helmholtz coils (diameter 200 cm and 180 cm) that were used to manipulate the vertical as well as the horizontal component of the MF, thus providing the possibility of modulating the inclination and intensity of the MF selectively. The coils were fed with a Coutant GPE 1000/12 d.c. source. Magnetic field intensity induced by switching the power supply on and off was below 500 nT.

To exclude directional information from optical stimuli the glass cuvette was covered with white cardboard (on its top, bottom and side walls). The intensity of illumination inside the glass cuvette was 200 lx.

During the experimental period the tadpoles were observed with a video camera (National WW-361) fitted with a time generator and tape recorder (National NV 8030). Following the test the angle of body axis orientation was measured from a video monitor by single-frame analysis using a protractor mounted on the monitor screen. Determinations of the angle of body axis were performed at intervals of 2 min. For the measurement of angles, the horizontal plane was used as the reference axis. Only those animals that were heading either parallel or antiparallel to the magnetic N–S direction were used for measurements.

The containers with larvae, Helmholtz coils and camera were located in a wooden chamber (2.60 m in height, 3.00 m in width and 2.70 m in length). The power supply for the coils and video recording unit was positioned outside the chamber 2 m away from the coils. A diagram of the experimental apparatus is shown in Fig. 1.

Three experimental series were performed with intact animals by changing the angle of inclination of the natural MF (65° in Würzburg, total intensity 42 000 nT) to 0° , 35° and 90° . Simultaneously the intensity was amplified to 75 000 nT. Experiments with sham-operated and operated tadpoles were made exclusively with an artificial dip of 35° . MF intensity was checked using a Bell 620 gaussmeter and a Hall detector. Corresponding determinations of dip were performed with an inclinometer.

Each series consisted of six experiments, except in the case of sham-operated animals and larvae tested 1 week after transection of the optic nerve (four tests only). In one set of experiments (50% of each series) the animals were first

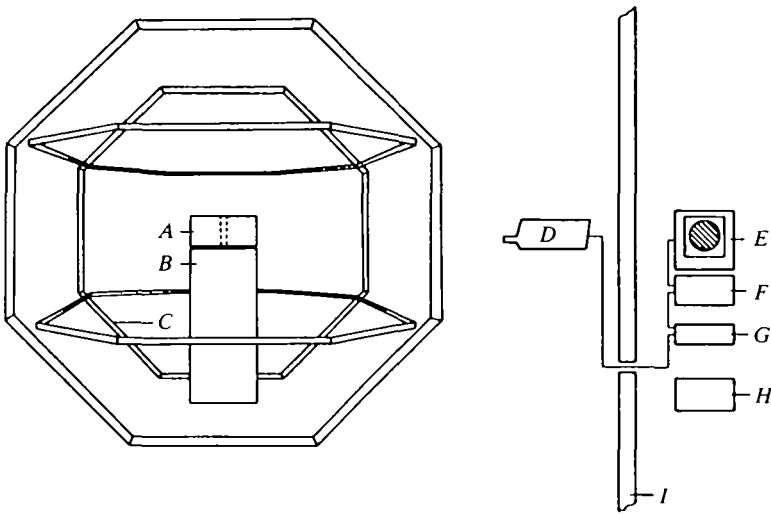


Fig. 1. Diagram of experimental apparatus viewed in the N-S direction. A, cuvette with tadpoles (stippled lines) and cardboard cover; B, wooden support; C, Helmholtz coil; D, video camera; E, video monitor with protractor; F, recording unit; G, time generator; H, power supply; I, wall of wooden chamber. The wiring between the Helmholtz coils and the power supply is not shown.

observed for 45 min under natural MF conditions, followed by a period of 45 min in the artificial MF, and then again for 45 min under normal MF conditions. In a second set of experiments the sequence of natural and artificial MF stimuli was inverted. To avoid rhythmic phenomena, tests were performed at the same time on consecutive days between 14:00 h and 16:15 h. The tadpoles were transferred to the cuvette at least 1 h before the beginning of each experiment.

Transection of the optic nerve

The animals were anaesthetized in a solution of 0.5×10^{-4} (v/w) of MS222 (Sandoz, Switzerland). The N. opticus was cut about 2 mm proximal to the eyes. Following the operation, the tadpoles were allowed to recover in a container with tap water for 24–48 h or, in a second series, for 7–8 days. Sham-operated animals were treated in the same way without transection of the optic nerve, and tested 24–48 h later.

Successful transection of the optic nerve was checked by testing the ability of operated tadpoles to adapt to their background. Intact animals aggregate or disperse their dermal melanophores when standing above a bright or dark background, respectively. The response of the pigment cells in the head region is thought to be exclusively controlled by optical inputs from the eyes. The adaptational response of animals was tested by placing them for 1 h over a white, then a black and finally a white background. Melanophore dispersion was assessed by using the melanophore index of Hogben and Slome (1931). Intact animals

displayed a significant adaptational response, whereas operated tadpoles consistently failed to show background adaptation.

Results

Fig. 2 illustrates the collected results of experiments with intact animals when the angle of inclination of the MF was changed to 90°, 35° or 0° with respect to the horizontal. The left-hand side of the diagram illustrates the results of those measurements in which the artificial stimulus had been given first, followed by the control situation (i.e. 65° inclination) and finally a period of artificial MF. The results of the inverse stimulus sequence are given on the right-hand side of the diagram. The corresponding data are summarized in Table 1 (first column). The

Table 1. *Collected results of all experiments*

		Total			N-S			S-N		
		EMF	AMF	D	EMF	AMF	D	EMF	AMF	D
90°	Mean	52.12	54.91	+2.79	53.71	56.40	+2.69	50.47	53.42	+2.95
	s.d.	11.91	11.44		11.22	11.32		12.38	11.36	
	s.e.m.	0.57	0.55		0.75	0.76		0.84	0.77	
	N	442	438		225	219		217	219	
35°	Mean	53.49	49.75	-3.84	54.89	50.32	-4.56	52.18	49.08	-3.10
	s.d.	10.81	12.54		10.77	13.38		10.67	11.43	
	s.e.m.	0.50	0.59		0.71	0.85		0.68	0.80	
	N	472	452		228	246		244	206	
0°	Mean	56.18	53.52	-2.66	58.17	55.29	-2.89	53.87	51.87	-2.00
	s.d.	12.85	12.63		12.80	12.83		12.53	12.21	
	s.e.m.	0.61	0.56		0.83	0.82		0.88	0.76	
	N	443	501		238	242		205	259	
35° Sham	Mean	58.86	54.39	-4.47	59.48	54.74	-4.74	58.26	54.06	-4.00
	s.d.	10.38	11.08		10.80	11.68		9.91	10.48	
	s.e.m.	0.61	0.67		0.94	1.02		0.82	0.89	
	N	290	270		143	131		147	139	
35° Cut 1	Mean	53.24	53.34	+0.10	53.39	52.06	-0.67	53.08	54.51	+1.59
	s.d.	11.42	11.56		11.21	11.38		11.65	11.60	
	s.e.m.	0.51	0.51		0.69	0.71		0.75	0.71	
	N	504	523		265	255		239	268	
35° Cut 2	Mean	54.51	54.18	-0.33	55.25	55.85	-0.60	53.77	52.61	-1.16
	s.d.	11.97	11.80		12.41	11.62		11.26	11.56	
	s.e.m.	0.69	0.74		0.99	1.00		0.92	1.07	
	N	305	253		156	134		149	119	

Total, mean angles of body axis orientation of all animals; N-S, mean of animals heading north; S-N, mean of animals heading south; EMF, natural magnetic field; AMF, artificial magnetic field; D=AMF-EMF; Mean, mean angle of body axis orientation; s.d., standard deviation; s.e.m., standard error of mean; N, number of measurements; 90°, 35° and 0°, angle of artificial dip; sham, sham-operated animals; cut 1, animals tested 24-48 h after operation; cut 2, animals tested 7-8 days after operation.

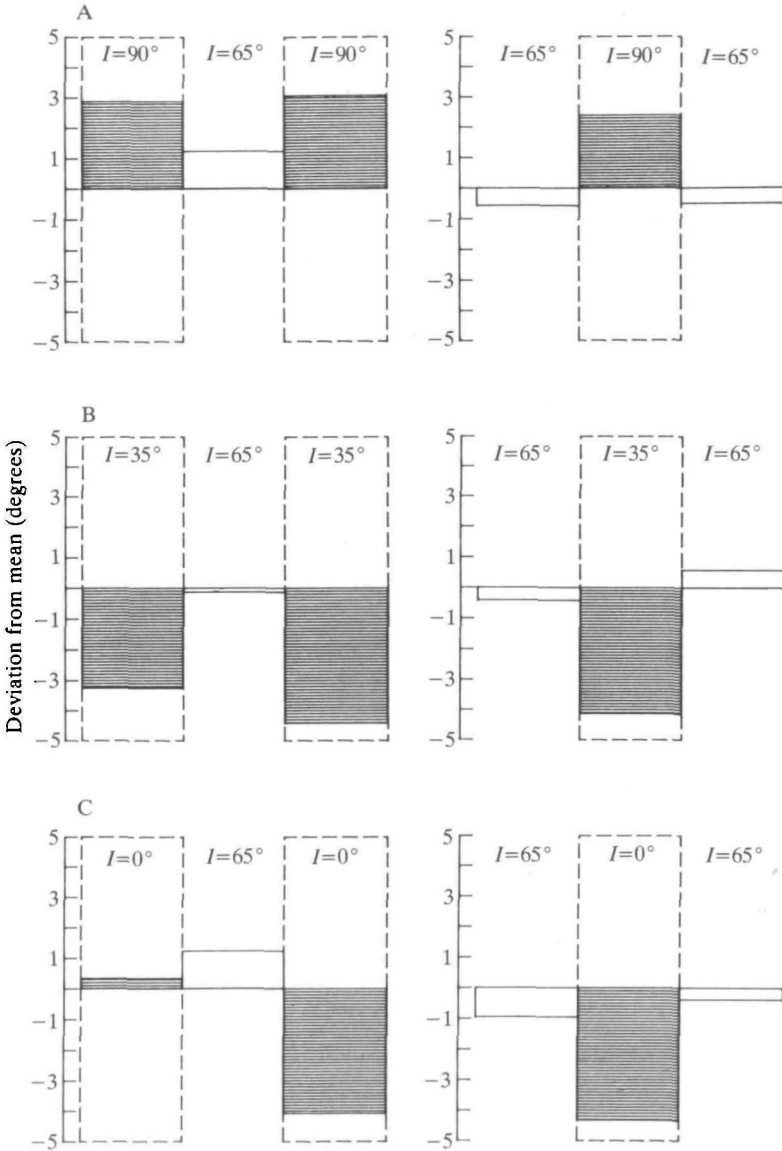


Fig. 2. Collected results obtained with intact animals ($N=270$) under different inclinations of the magnetic field (MF) (A 90° ; B 35° ; C 0°). Left-hand side of the diagram, results obtained when applying the artificial MF stimulus first, followed by a control period in the natural MF, and finally by the artificial MF. Right-hand side of the diagram, inverse sequence of MF stimuli. Each bar represents measurements obtained during a 45 min exposure to an altered MF or to the natural MF. Ordinate, angular deviation of body axis orientation from the mean of controls. Hatched columns in stippled rectangles, results in the artificial MF. Open columns, results in the natural MF.

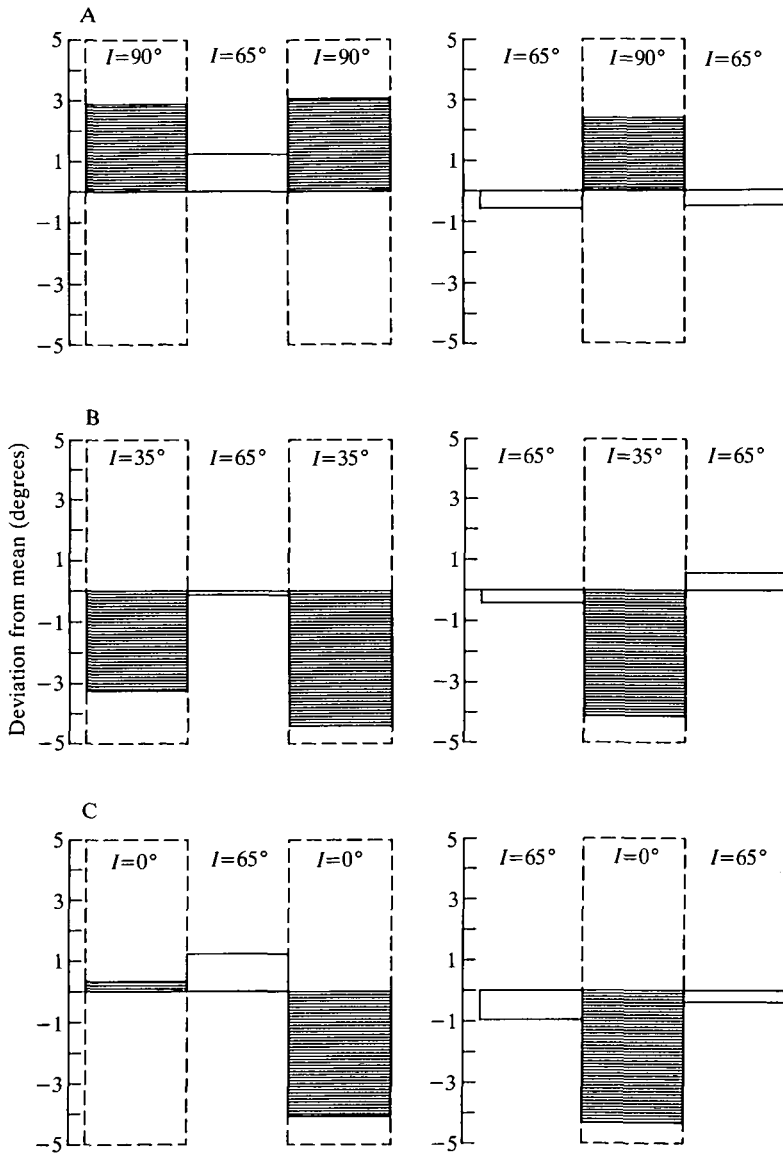


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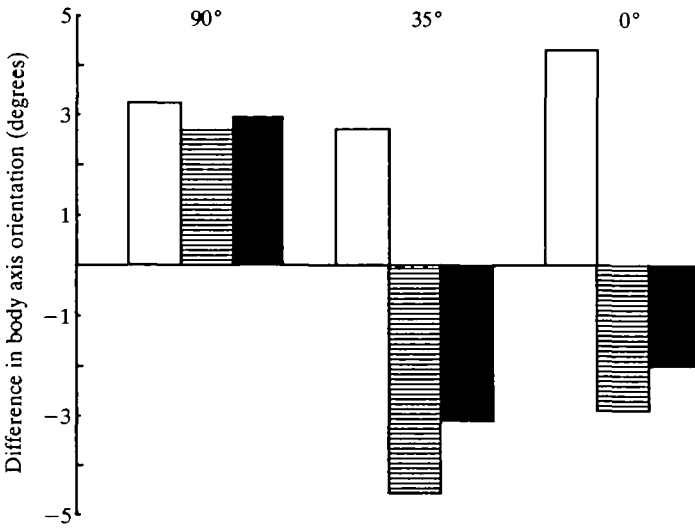


Fig. 3. Differences in body axis orientation between tadpoles heading north and tadpoles heading south. Open bars, angular difference between controls in the ambient MF heading north and south [EMF(N-S)–EMF(S-N)]; see Table 1. Hatched bars, angular differences between experimental animals heading north and corresponding controls [EMF(N-S)–AMF(N-S)]. Solid bars, angular differences between experimental animals heading south and corresponding controls [EMF(S-N)–AMF(S-N)].

different artificial MF situations consistently caused a significant deviation of body axis orientation. A change of the angle of inclination from 65° to 35° caused a corresponding deviation of the mean angle of body axis orientation of 3.84° (t -test: $P < 0.0001$) compared with controls. Similar results were obtained when the vertical component of the MF was compensated, accompanied by a simultaneous amplification of the horizontal MF vector, thus producing a field of 0° inclination. These larvae showed a deviation of 2.66° ($P < 0.002$). The failure to produce an effect in the first period of artificial MF inclination (see Fig. 2C, on the left) remains unexplained. When the animals were subjected to an MF with an inclination angle of 90°, they responded with an inverse reaction, i.e. they increased their angle with respect to the horizontal. The results of this experimental series show that gravity-oriented behaviour in the larvae of *Xenopus laevis* is subject to the influence of a steady MF of low intensity.

Since the larvae were free to move inside the container, they could align their body axis in the N–S as well as in the S–N direction. With regard to the parallel or antiparallel alignment to the magnetic N–S direction, the data show no significant differences in the angular deviation caused by the artificial MF (Fig. 3). However, comparison of control measurements in the natural MF within each of the three experimental series with intact animals shows that the tadpoles consistently maintained a larger angle of body axis orientation when heading in the N–S than in the opposite direction.

Fig. 4 compares the experimental results obtained with intact tadpoles with

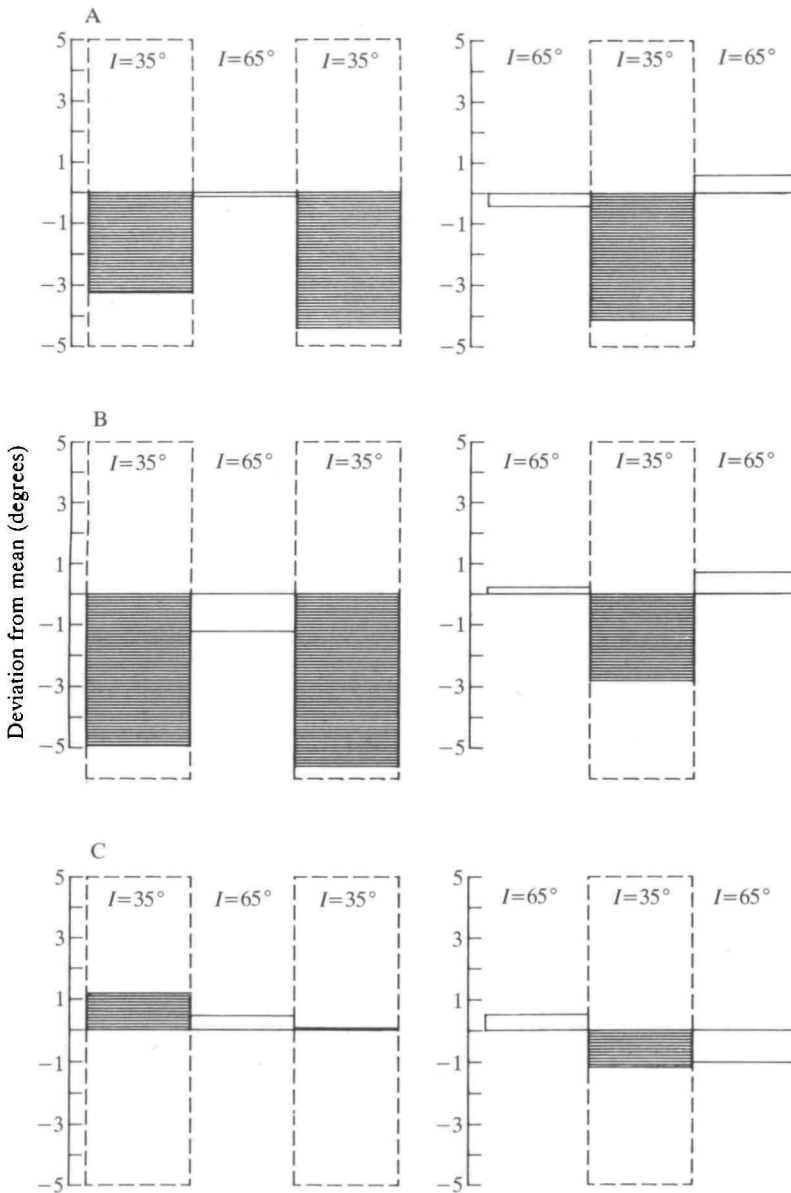


Fig. 4. Collected results obtained with an artificial inclination of 35° in intact animals (A), sham-operated larvae (B) and tadpoles with transected optic nerves tested 24–48 h after the operation (C). For further explanation see Fig. 2.

those obtained from animals with transected optic nerves. The test stimulus was an artificial dip of inclination to 35° . For comparison, Fig. 4A shows the results in intact animals and Fig. 4B illustrates the behaviour of the sham-operated animals. No significant differences between these groups can be seen. Fig. 4C shows the results of measurements obtained with animals in which the optic nerves had been

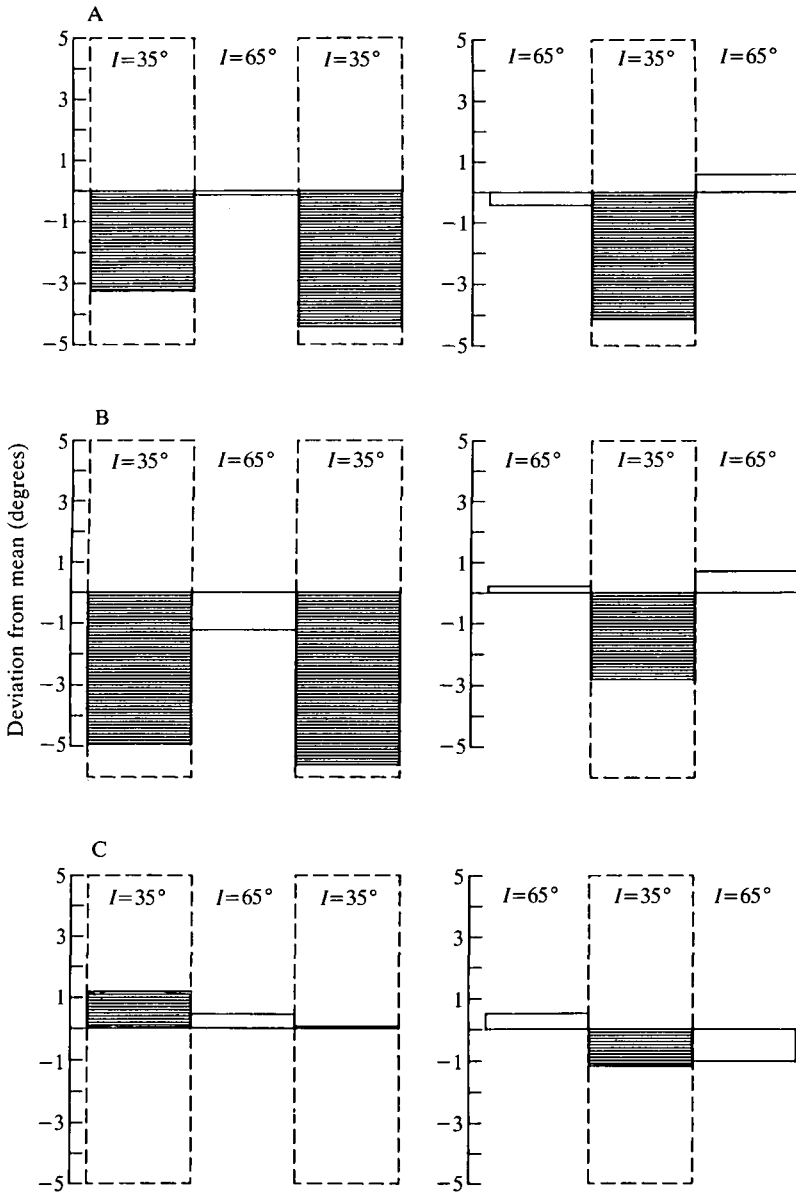


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cut 24–48 h before the test. Experiments with animals tested 7–8 days after the operation gave similar results (see Table 1). Thus, no behavioural reaction to the applied artificial MF was observed following the functional elimination of the peripheral optical system.

Discussion

The present study confirms earlier demonstrations of the ability of *Xenopus laevis* tadpoles to perceive MFs (Leucht, 1987; Imgrund *et al.* 1989) and shows that the mechanisms regulating gravity-oriented behaviour can be affected by MF. This influence of a weak MF has been reported predominantly at the behavioural level (Martin and Lindauer, 1977). Evidence for direct involvement of gravity receptors has come only from electrophysiological experiments in honeybees (Korall and Martin, 1987), showing variations in impulse frequency due to altered intensities of the horizontal component of the natural MF.

The weak response to the applied MF conditions shows that gravity is the main source of information for the alignment of the tadpole's body axis. However, the MF seems to have a modulatory effect on this orientational behaviour, similar to the MF-induced misdirection observed in dancing honeybees (Lindauer and Martin, 1968).

The experimental results suggest that the effect of the artificial MF is triggered by its total intensity and its corresponding angle of inclination. If the tadpoles were to rely on the horizontal or vertical component of the MF, their response (i.e. their angle of body posture) would be symmetrical when comparing animals heading north or south. This is not the case, as can be seen from Fig. 3. When observed in the natural MF the animals heading north consistently maintained a steeper angle of body axis orientation than those directed antiparallel. In addition, the response to artificial inclination of the MF to 35° and 0° was more pronounced in animals heading north. The results indicate that both the intensity and the polarity of the total MF component can influence the strength of the behavioural response. When the angle of inclination is artificially increased to 90°, producing a magnetic situation that is symmetrical for tadpoles heading to the north or the south, no difference in the MF-induced variation of the angle of body axis orientation was observed.

The finding that tadpoles with transected N. opticus do not react to the applied MF supports the idea of the involvement of the peripheral optical system in MF reception. The data are in accordance with the observation that the optically triggered mechanism of background adaptation can be influenced by MF in *X. laevis* (Leucht, 1987) and add another element to the list of existing examples in vertebrates (Cremer-Bartels, 1984; Olcese *et al.* 1985) of the participation of optical systems in MF reception. Olcese *et al.* (1985) found that blinding Sprague–Dawley rats by transecting the optic nerve resulted in the abolition of MF-induced changes in pineal melatonin synthesis. More recently, Olcese *et al.* (1988) observed reductions of dopamine and norepinephrine levels in the retinas of rats

resulting from alteration of the ambient MF. This effect was abolished in animals with damaged photoreceptors. In the blowfly *Calliphora vicina* Phillips (1987) found responses of photoreceptors to MF by using intracellular recording techniques. Despite the demonstration of the involvement of optical systems in MF detection, these observations give no answer to the question of whether light itself is a prerequisite for the reception of MF.

The results also indicate that the sensory structures responsible for gravity perception are not directly affected by the applied MF stimulus. Rather, it can be assumed that the motor outputs related to gravity information are modulated neuronally by information coming from the optical system. Multimodal processing of sensory inputs (i.e. interaction of light and gravity with MF) has been described in the honeybee (Leucht, 1984) and in the pigeon (Semm *et al.* 1984).

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