Magnetic Discrimination Learning in Rainbow Trout (*Oncorhynchus mykiss*)

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Although conditioning techniques are the most powerful way to study behavioural responses by animals to external stimuli, the magnetic sense has proved surprisingly resistant to conditioning approaches. This study demonstrated learned discrimination of magnetic field intensity stimuli by a new species, the rainbow trout (*Oncorhynchus mykiss*). In a unitary conditioned discrimination technique, four juvenile rainbow trout were trained to strike a target at the end of a response bar in anticipation of food. In successive experiments, the trout failed to discriminate the presence and absence of a vibration stimulus, but subsequently learned to discriminate the presence and absence of a magnetic field intensity anomaly (peak intensity of $75\,\mu$Tesla). The authors conclude that the necessary conditions for training animals to magnetic intensity are the use of spatially distinctive stimuli and of a conditioned response that requires movement.

1. INTRODUCTION. Intensive study of the mechanisms involved in animal orientation over the last half-century has resulted in the discovery of new sensory modalities, including the magnetic sense. Although evidence for the existence of the magnetic sense has come over the last 25 years from orientation experiments with many species (for a review see: Wiltschko and Wiltschko, 1995), the sense remains mysterious at least in part because conditioning experiments have so often failed.

Most magnetic field conditioning experiments have attempted to train homing or migratory species to respond to magnetic field direction: first, because a magnetic sense seems likely to be best developed among such species; and, secondly, because magnetic field direction is known from orientation experiments to be biologically important. Paradoxically, however, such attempts have produced mostly negative or at best controversial results; whereas honey bees (*Apis mellifera*) (Walker and Bitterman, 1985; Kirschvink and Kirschvink, 1991) and yellowfin tuna (*Thunnus albacares*) (Walker, 1984) readily learned to discriminate magnetic field intensity stimuli. The key conditions suggested for the success of these experiments in contrast to those that failed were the use of spatially distinctive stimuli and a conditioned behavioural response requiring movement.

As part of work aimed at developing a coherent understanding of the structure and function of the magnetic sense in a single vertebrate species, this study sought to demonstrate and analyse behavioural responses to magnetic field intensity stimuli in the rainbow trout (*Oncorhynchus mykiss*). Systematic investigation of behavioural responses to magnetic field intensity stimuli has previously been done for the honey bee (Walker and Bitterman, 1985, 1989*a*, *b*, 1991; Walker *et al.*, 1990),
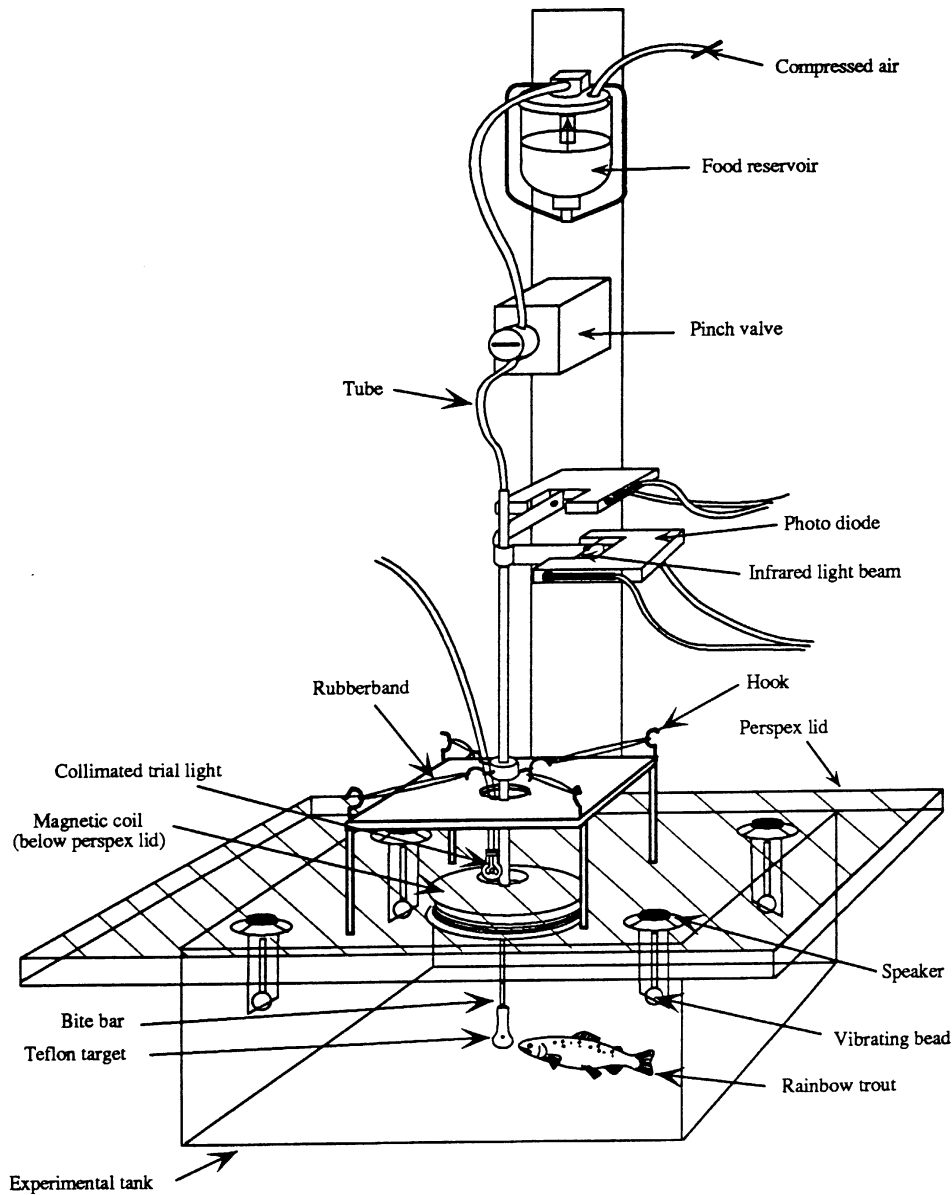
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Fig. 1. Schematic drawing of the experimental apparatus used for the recording of conditioned behavioural responses to vibration and magnetic field intensity stimuli by individually trained rainbow trout. (Note: not drawn to scale).

but has not been attempted for a vertebrate because a suitable model species has yet to be identified.

When choosing a model species for magnetic field conditioning work, it will be important to optimise the mixture of advantages and disadvantages any particular species will bring to the experimental work. The magnetic sense is
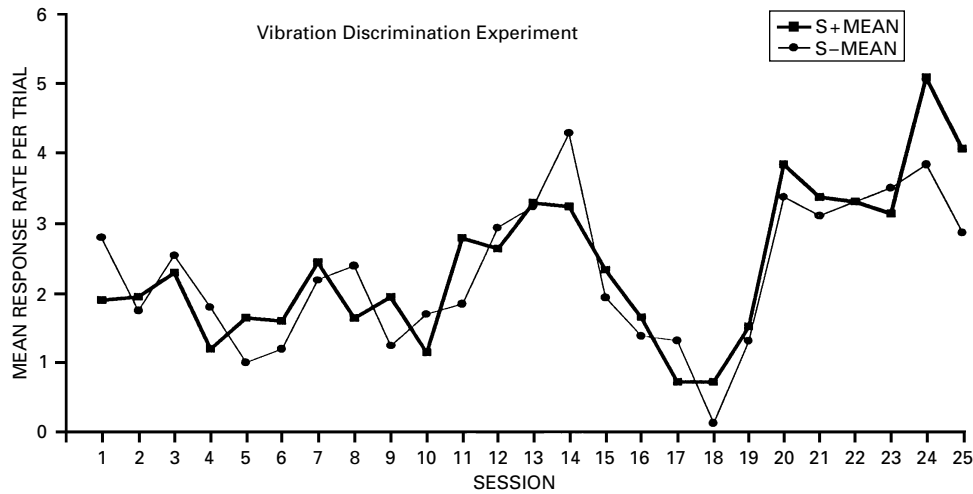
Fig. 2. Discrimination of the presence and absence of a free-field dipole vibration stimulus by four individually trained rainbow trout. Presented are the overall mean response rates per 15 s reinforced (S+) trial and a 15 s unreinforced (S−) trial with the data being blocked over the 5 S+ trials and the 5 S− trials given in each session.

likely to be well developed in a marine pelagic species, such as yellowfin tuna, but such species are not at all well suited to laboratory conditions (Walker, 1984). Conversely, goldfish (*Carassius auratus*) are easy to maintain and work with under laboratory conditions and their discrimination learning has been studied in great detail. Goldfish are not known, however, to migrate or home over long distances and an early attempt to train them to discriminate magnetic field intensity stimuli was unsuccessful (Walker and Bitterman, 1986).

Rainbow trout (*Oncorhynchus mykiss*) offer a suitable compromise between the extremes of the tuna and the goldfish as an experimental species. New Zealand stocks of rainbow trout are derived from the migratory steelhead variety of this species (Stokell, 1955). Also, trout are readily available and easily maintained in the laboratory situation. Evidence suggesting the existence of a magnetic sense in trout comes from previous behavioural studies indicating the use of a magnetic compass (Chew and Brown, 1989). These qualities of rainbow trout gave the authors confidence that it would be possible to train trout to discriminate magnetic field intensity stimuli in the same way as has been previously done in tuna while achieving the same degree of experimental control expected for work with goldfish.

2.   METHODS AND MATERIALS

2.1. *Experimental apparatus*. The experimental apparatus used in this study was designed to permit close association in space and time of stimulation, behavioural response, and reinforcement (Fig. 1). The apparatus was constructed atop a perspex sheet to be placed during discrimination sessions over the experimental tank of an individual fish. Liquid fish food situated in a reservoir at the top of the apparatus was pumped through a hollow metal response bar. The

response bar, tipped with a white teflon target (25 mm length), projected vertically to just below the water surface at the centre of the experimental tank. A small opening in the target allowed the liquid food to be delivered to the fish as positive reinforcement when the fish hit the target during discrimination sessions. All experimental procedures were fully automated.

2.2. *Vibration discrimination experiment*. To provide comparative data, the trout were first tested for response to a vibration stimulus. A free-field dipole vibration stimulus was generated using four vibration probes, each consisting of a small speaker modified with a thin wood stick capped with a 15 mm diameter wooden bead. The probes were situated in the four corners of the experimental tank. Each probe was operated at the maximum available amplitude by a 40 Hz sine wave signal, a frequency previously shown to stimulate the trout's mechanosensory lateral line system (Wubbels *et al.*, 1993).

A two-group balanced experimental design was used to control for possible generalised effects of the stimulus on behaviour. The presence of the free-field dipole vibration stimulus became the reinforced stimulus (S+) for two fish and its absence the non-reinforced stimulus (S−). For the other two fish the stimulus contingencies were reversed. That is, S+ and S− were the absence and presence of the vibration stimulus respectively. Vibration discrimination data were collected over a period of 25 sessions.

2.3. *Magnetic discrimination experiments*. A 50-turn magnetic coil (4 cm in diameter), whose axis was aligned with the response bar, was mounted underneath the perspex lid directly above the water surface. A direct current of $3 \cdot 0$ Å through the coil induced a non-uniform, vertical magnetic field intensity anomaly focussed at the target with a peak intensity of about 75 micro Tesla (μT) (about $1\frac{1}{2}$ times Earth-strength) and superimposed on the local Earth background field of about 55 μT.

To control for possible generalised effects on behaviour of the artificial field, the experimental design was again balanced using two groups of fish. The altered field became the reinforced stimulus (S+) for two fish and the uniform background field became the non-reinforced stimulus (S−). For the other two fish, S+ and S− were the background and altered fields respectively.

After 15 sessions the reinforced contingencies were reversed for a second magnetic discrimination experiment, with S+ now being the uniform background field and S− the altered field for the first group of trout, while S+ became the altered field and S− the uniform background field for the second group. That is, fish that had been previously rewarded for response to the presence of the magnetic field intensity anomaly were now rewarded in its absence and *vice versa*. Responses during this reversal experiment were recorded over a period of 20 sessions.

2.4. *Discrimination training procedures*. The unitary conditioned discrimination technique used in this study was adapted from the one previously successfully applied to magnetic discrimination learning in yellowfin tuna (*Thunnus albacares*) (Walker, 1984). Four juvenile rainbow trout (fork length 15 cm) were trained individually to swim to and strike repeatedly at the teflon target in order to obtain food. The measure of behaviour used to detect discrimination of the

presence and absence of the free-field dipole vibration stimulus and later of the magnetic field intensity anomaly was thus the rate at which the fish struck the target in anticipation of reinforcement or non-reinforcement with food at the end of the trial (Woodward and Bitterman, 1974).

Discrimination training sessions comprised ten 15-second trials, five S+ and five S−, presented in balanced quasi-random order (Gellerman, 1933). Stimulus reinforcement consisted of a food reward being delivered at the first response following the end of each S+ trial. The punishment for responding to the unreinforced stimulus (S−) was a time penalty which extended S− trials without the possibility of food being obtained. At the end of each S− trial, a 20-second penalty timer started, which was reset by each subsequent response until the subject either failed to respond for 20 seconds or a total of 60 seconds of penalty time was accumulated. This procedure therefore applied an energy cost to continued responding. Between trials, the tank was darkened for a variable interval (average length 120 seconds).

3. RESULTS. Conditioned discrimination is a technique based on the principles of instrumental conditioning and *discrimination* is defined as differential responsiveness to different stimuli (Harré and Lamb, 1986). Animals which do not initially respond differently to different stimuli can often learn to do so through reinforcements in the form of reward and/or penalty. Such a change in behaviour as a result of experience is commonly referred to as learning. If the trout learn to discriminate the presence and absence of the stimulus presented, it is expected that the response rates to S+ and S− trials change relative to each other with time. However, if no discrimination learning occurs, the two rates are not expected to separate from one another.

All discrimination data are presented as the mean response rate per trial by four individually trained fish with the data blocked over the five S+ and S− trials given in each training session. The results were statistically analysed with a three-way mixed-module Analysis of Variance (ANOVA) as well as Tukey's Studentised Range (HSD) Tests on all main effects.

3.1. *Vibration discrimination experiment.* The experimental subjects failed to discriminate the presence and absence of the free-field dipole vibration stimulus as no clear and persistent separation of the mean response rate to S+ and S− could be detected (Fig. 2). Rather the two response rates varied closely together throughout the 25 consecutive sessions given, and were relatively low and variable when compared with those of the two magnetic discrimination experiments. This non-separation of the S+ and S− response rates was confirmed statistically by a non-significant ANOVA result ($F_{1,3}$ stimuli = 0·1092, $P = 0·7628$) and further validated a by non-significant stimuli main effect in Tukey's Test. Also, all effects regarding a change of S+ and S− response rates with time throughout the discrimination experiment, which thus indicate discrimination learning, were found to be non-significant (ANOVA; $F_{19,57}$ stimuli × sessions = 1·4992, $P = 0·1537$; $F_{42,480}$ subjects × stimuli × sessions = 0·9653, $P = 0·5360$). However, overall mean response rates were highly variable among individual fish as well as over individual sessions (ANOVA: $F_{3,4}$ subjects = 6·4214, $P = 0·0045$; $F_{3,4}$ sessions = 1·9440, $P = 0·0488$; $F_{3,4}$ subjects × sessions = 4·0482, $P = 0·0001$).

The non-separation of the S+ and S− response rates occurred no matter whether the food reward was associated with the presence of the vibration stimulus in one trout group or with its absence in the second group.

3.2. *Magnetic discrimination experiments.* After an initial learning phase of about three sessions during which the fish did not distinguish the two magnetic stimuli, a clear difference in response rates developed with the subjects producing a consistently higher rate of response to the reinforced stimulus (S+) than to the non-reinforced stimulus (S−) (Fig. 3). The difference between the average
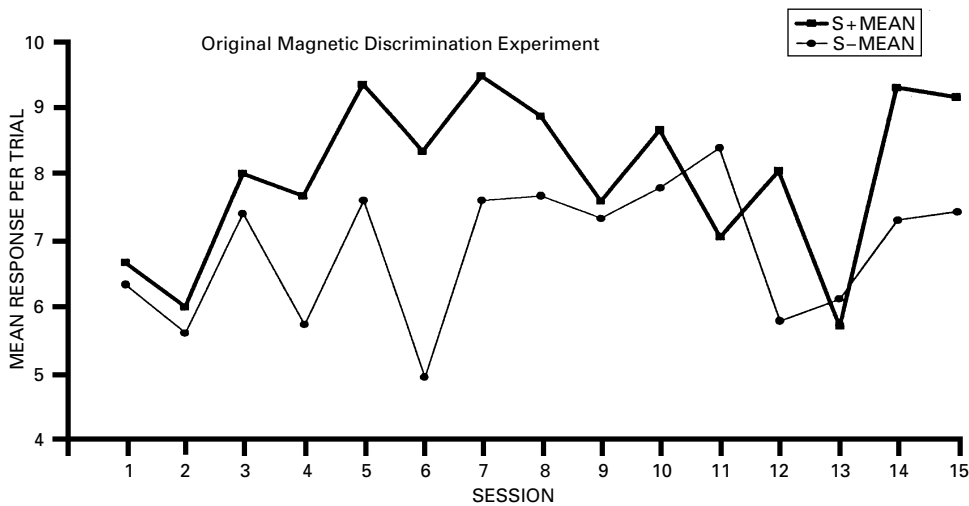

Fig. 3. Discrimination of the presence and absence of a magnetic field intensity stimulus by four individually trained rainbow trout. Presented are the overall mean response rates per 15 s reinforced (S+) trial and 15 s unreinforced (S−) trial with the data being blocked over the 5 S+ trials and the 5 S− trials given in each session.

response rates to S+ and S− was statistically reliable (ANOVA; $F_{1,3}$ stimuli = 26·7272, $P = 0·0140$). Graphs for individual fish (not shown) were all similar to Fig. 2 but varied in overall mean response rate together with sharpness and timing of onset of the discrimination (ANOVA: $F_{3,5}$ subjects = 40·5086, $P = 0·0005$). In particular, response to S− was more variable among individual fish than response to S+ . Stimuli and subjects main effects were confirmed by statistically significant comparisons with Tukey's Test. The separation of the S+ and S− response rates with time during the experiment was also confirmed statistically (ANOVA: $F_{42,480}$ subjects × stimuli × sessions = 1·5948, $P = 0·0122$). The likely source of this effect is interaction between the development of the discrimination through learning (response rates to S+ and S− changed with time during the experiment) and variability of behaviour among the subjects. The difference in the S+ and S− response rates occurred no matter whether the food reward was

associated with the presence of the magnetic field intensity anomaly in one trout group or with its absence in the second group.

In the second discrimination experiment, the reversal of the reinforcement contingencies for the two groups resulted initially in a phase of about eight sessions during which the trout gained experience with the new experimental situation and therefore did not distinguish between the two magnetic stimuli. However, thereafter the mean S+ response rate significantly rose above the mean S− response rate for the remaining sessions. That is, the fish learned to respond at a higher rate to the stimulus that had previously been unrewarded and at a lower rate to the previously rewarded one (Fig. 4). In graphs for individual fish,
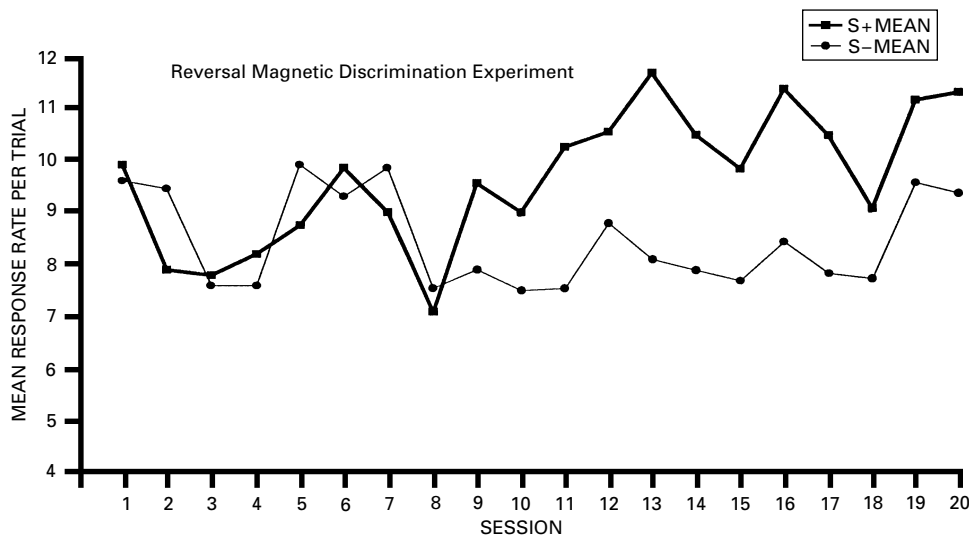

Fig. 4. Reversal discrimination of the presence and absence of a magnetic field intensity stimulus by four individually trained rainbow trout. Presented are the overall mean response rates per 15 s reinforced (S+) trial and 15 s unreinforced (S−) trial with the data being blocked over the 5 S+ trials and 5 S− trials given in each session.

the overall mean response rates together with sharpness and timing of onset of the discrimination again varied between individual fish ($F_{3,4}$ subjects $= 7·6279$, $P = 0·0331$), but not as strongly as in the first magnetic discrimination experiment. The absence of a significant stimuli main effect in the ANOVA ($F_{1,3}$ stimuli $= 4·2083$, $P = 0·1326$) is most likely caused by the closeness of the two response rates during the initial learning phase statistically cancelling out the clear difference in response rates in the latter half of the experiment. A statistically significant difference between the mean S+ and S− response rates was, however, demonstrated by Tukey's Test, which in contrast to ANOVA is generally quite robust in its assumptions. Furthermore, the separation of the S+ and S−

response rates with time during the experiment was confirmed statistically ($F_{19,57}$ stimuli × sessions = 2·5156, P = 0·0038), thus indicating the development of the discrimination through learning. The reversal of the magnetic discrimination learning occurred in both groups of fish.

In conclusion, a significant difference between the two response rates developed in the course of both magnetic discrimination experiments, with the S+ rate being significantly and persistently greater than the S− response rate even after a reversal of the reinforcement contingencies. These results, both graphically and statistically, are in sharp contrast to the discrimination results obtained for the free-field dipole vibration stimulus.

4. DISCUSSION. The results presented in this study clearly establish magnetic sensitivity in a new species, the rainbow trout (*Oncorhynchus mykiss*), thus adding it to a growing list of magnetosensitive animals. They also demonstrate the importance of appropriate choice of experimental conditions in magnetic conditioning experiments.

4.1. *The magnetic sense in rainbow trout (Oncorhynchus mykiss).* Discrimination is defined as differential responsiveness to different stimuli (Harré and Lamb, 1986). An animal can respond differentially to different stimuli only when it can recognise the difference between them. Such discrimination ability is only possible if the animal possesses the appropriate sensory system. Consequently, for any given conditioning technique, the animal's behaviour would be expected to change as the result of discrimination learning in a similar fashion for all sensory stimuli that the animal can detect.

Instead of providing an example of a successful discrimination performance with which the results of the magnetic discrimination experiments could have been compared, the failure of the trout to respond differentially to the presence and absence of the free-field dipole vibration stimulus gave a clear indication of what the trout's conditioned behaviour should look like when the trout could not discriminate the stimulus. Aposteriori characterisation of the free-field dipole vibration stimulus indicated that the net currents of waterflow as well as the dipole particle motion of the water medium itself at a distance of more than a few centimetres from the vibration probes were well below the physiological sensitivity threshold of the trout's lateral line system.

The only possible conclusion from the successful discrimination of the presence and absence of the magnetic field intensity anomaly in the first magnetic discrimination experiment is that rainbow trout do indeed possess a magnetic sensory system. The temporary fading of the overall discrimination performance in Sessions 9–13 was most likely caused by the negative experience of three of the four trout previously tested using the vibration stimulus. The individual discrimination performance of the fourth trout, which replaced one fish after the vibration discrimination experiment, was more stable than that of the other three fish and showed no fading effect over the course of the experiment.

The successful reversal of the discrimination learning, the increased separation of the two response rates, and the absence of a fading effect in the second magnetic discrimination experiment provided further evidence for a magnetic sense in rainbow trout. That is, this species is not only able to learn magnetic

discrimination and improve its performance with increased experience, but is also capable of modifying that conditioned behaviour according to changes in the reinforcement contingencies.

Although discrimination of magnetic intensity anomalies has been demonstrated so far for only two fish species, yellowfin tuna and rainbow trout, the results presented here fit comfortably with a large body of comparative data on discrimination of different stimuli by other vertebrates. Equivalent discrimination performance independent of whether the magnetic intensity anomaly was the reinforced or the non-reinforced stimulus and the improvement of performance after reversal of the reinforcement contingencies are well known phenomena in vertebrate learning (Bitterman, 1984). After allowing for differences in the conditioned response used, the discrimination performance achieved by the trout after reversal is comparable with that achieved by goldfish in a red–green colour reversal discrimination experiment (Woodard and Bitterman, 1974), a species which has provided some of the best conditioned discrimination results available so far. A clear conclusion from the study presented here, then, is that the magnetic sense of rainbow trout can be studied in the same way as other sensory modalities.

4.2. *Conditions required for successful magnetic conditioning*. The results presented here are consistent with the idea that animals learn most readily to discriminate magnetic fields when the magnetic field stimuli are spatially distinctive, and the conditioned response requires movement. Yellowfin tuna, honeybees, and now rainbow trout all readily discriminated magnetic fields under these two experimental conditions. However, the tuna and honeybee both failed to discriminate when one of these conditions was not met (Walker, 1984; Walker *et al.*, 1989). Consequently, failures to condition animals to magnetic intensity stimuli (Wiltschko and Wiltschko, 1995; 1996; Kirschvink, 1989; Griffin, 1982) may be explained by at least one of these conditions not being met in the experimental design.

The work presented here places the relative ease with which animals learn to discriminate magnetic intensity anomalies in contrast with the difficulties experienced in training animals to respond to magnetic field direction, (Wiltschko and Wiltschko, 1995, 1996). This contrast is heightened by the clear understanding of magnetic compass orientation (for a review see: Wiltschko and Wiltschko, 1995) compared with the suggested uses of magnetic field intensity and magnetic anomalies in long distance orientation (Kirschvink and Gould, 1981; Gould, 1982; 1985; Kirschvink and Walker, 1985). One explanation put forward for this general failure is a fundamental limitation of conditioning techniques in demonstrating compass mechanisms due to their inevitable spatial restrictions by an experimental apparatus and the question of pairing directional responses with a food reward (Wiltschko and Wiltschko, 1996). The authors suggest the alternative hypothesis that the necessary experimental conditions for training animals to respond to magnetic field direction have not yet been achieved. This hypothesis can be tested by a systematic exploration of parameters of conditioning experiments, such as the response to be conditioned, attention to the stimulus by the animal and motivation of the animal to respond to magnetic

direction. A successful outcome of such a systematic study could be expected to underpin new developments in the study of magnetic navigation.

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KEY WORDS

1. Animal training.   2. Magnetic stimuli.   3. Rainbow trout.