Author: S. Denzau, D. Kuriakose, R. Freire, U. Munro and W. Wiltschko

Title: Conditioning domestic chickens to a magnetic anomaly

Journal: Journal of Comparative Physiology A **ISSN**: 0340-7594 1432-1351

Year: 2011

Volume: 197

Issue: 12

Pages: 1137-1141

Abstract: Young domestic chicks of two strains, ISA brown layers and White Leghorn X Australorps, were trained to associate a magnetic anomaly with food. This was done by feeding them in their housing boxes from a dish placed above a small coil that produced a magnetic anomaly roughly six times as strong as the local geomagnetic field. Unrewarded tests began on day 9 after hatching. In a square arena, two corresponding coils were placed underneath two opposite corners. One coil, the control coil, was double-wrapped producing no net magnetic field, while the other in the opposite corner produced a local magnetic anomaly similar to that experienced during feeding. The chicks favoured the corner with the anomaly from day 10 after hatching onward. Both strains of chickens showed this preference, indicating that they could sense the local changes in the magnetic field.

URLs: http://dx.doi.org/10.1007/s00359-011-0675-0 ; http://researchoutput.csu.edu.au/R/-?func=dbin-jump-full&object_id=32951&local_base=GEN01-CSU01

Author Address: rfreire@csu.edu.au

CRO Number: 32951

Conditioning domestic chickens to a magnetic anomaly

Susanne Denzau[1], Dany Kuriakose[1], Rafael Freire[2], Ursula Munro[3], Wolfgang Wiltschko[1]

[1] FB Biowissenschaften, J. W. Goethe-Universität Frankfurt, Siesmayerstrasse 70, D-60054 Frankfurt am Main, Germany,

[2] School of Animal and Veterinary Science, Charles Sturt University, Wagga Wagga, NSW 2678, Australia,

[3] The School of the Environment, Centre of Environmental Sustainability (CEnS), University of Technology, Sydney, Broadway, NSW 2007, Australia,

Correspondence

Wolfgang Wiltschko: Email: wiltschko@bio.uni-frankfurt.de

Abstract

Young domestic chicks of two strains, brown layers and white broilers, were trained to associate a magnetic anomaly with food. This was done by feeding them in their housing boxes from a dish placed above a small coil that produced a magnetic anomaly roughly six times as strong as the local geomagnetic field. Unrewarded tests began on day 9 after hatching. In a square arena, two corresponding coils were placed underneath two opposite corners. One coil, the control coil, was double-wrapped producing no net magnetic field, while the other in the opposite corner produced a local magnetic anomaly similar to that experienced during feeding. The chicks favoured the corner with the anomaly from day 10 after hatching onward. Both strains of chickens showed this preference, indicating that they could sense the local changes in the magnetic field.

Keywords: Domestic chicken, magnetic anomaly, orientation, conditioning experiments, magnetoreception

Introduction

Conditioning experiments are a useful tool to demonstrate the ability of animals to detect sensory cues of different nature. Chickens, for example, can be easily trained to respond to visual, acoustic and a variety of other stimuli (e.g. Salzen et al. 1971, Vallortigara and Andrew 1994, Vallortigara et al. 1998). However, training animals to magnetic stimuli – magnetic intensity as well as magnetic directions - has often been problematic: results indicating magnetic sensitivity were often questioned because they could not be replicated (for a review of early attempts to train animals to magnetic stimuli, see Wiltschko and Wiltschko 1995). Therefore many scientists remained sceptical about the ability of animals to sense the magnetic field, although its use in orientation and navigation had been demonstrated numerous time in spontaneous behaviours, such as bird migration or the orientation perpendicular to the shore in salamanders and beach-dwelling arthropods (for review, see Wiltschko and Wiltschko 1995).

Recently, several successful attempts of conditioning animals of various vertebrate taxa to magnetic stimuli have been reported (e.g. fish: Shcherbakov et al. 2005; mammals: Muheim et al. 2007). In birds, successful conditioning involved two different types of stimuli and approaches. One approach was to condition pigeons, *Columba livia* f. *domestica*, to use the presence and absence of a strong local magnetic anomaly as an indicator in a two-choice task (Mora et al. 2004) or to associate an anomaly with a food source (Thalau et al. 2007). The anomalies consisted of marked changes in magnetic intensity; the respective information is mediated by the magnetite-based receptors in the beak (Fleissner et al. 2003; Falkenberg et al. 2010, Wiltschko et al. 2010) and the ophthalmic branch of the trigeminal nerve (e.g. Semm and Beason 1990; Beason and Semm 1996; Mora et al. 2004; Heyers et al. 2010). The other approach involved conditioning birds to specific magnetic directions, using the tendency of young domestic chicks, *Gallus gallus*, and ducklings, *Anas platyrhynchos,* to search for an imprinting object (Freire et al. 2005; Wiltschko et al. 2007, Freire and Birch 2010) and of zebra finches, *Taeniopygia guttata* (Estrildidae), and pigeons to search for food (Voss et al. 2007; Wilzeck et al. 2010). The respective directional information is mediated by radical pair processes in the eye and the visual system (e.g. Wiltschko et al. 2007; Rogers et al. 2008; Keary et al. 2009; Zapka et al. 2010). That is, the different magnetic stimuli – intensity and direction – involve two different receptor systems (see Wiltschko and Wiltschko 2007).

When Freire et al. (2005) and Wiltschko et al. (2007) performed their experiments to analyse the magnetic compass in young domestic chickens, they rewarded the animals with a social stimulus – the imprinting object - which seemed to strongly motivate them to learn the task. However, not all chickens could be trained to show a positive response – one strain of chickens, white broilers, a synthetic line including Australorps, Sussex and White Leghorn, failed to respond, possibly due to an inability to detect magnetic directions (Freire et al. 2008). This led to the question whether a similar difference between strains exists in the ability to detect magnetic anomalies, where changes in intensity are the major component of the stimulus. We therefore modified the method used by Thalau et al. (2007) and trained young chickens of two strains closely related to those used by Freire et al. (2008) to associate a food source with a magnetic anomaly, testing for a preference of the corresponding anomaly in a test arena.

Methods

The experiments were performed in January and February 2009 in the laboratories of the Charles Sturt University, Wagga Wagga, NSW, Australia.

Test birds

The two original strains used by Freire et al. (2008) were no longer available, so we had to use two closely related strains instead. For the experiments in January, we used 44 male ISA brown layer chicks (Baiada Poultry, Marsden Park, NSW, Australia), a strain similar to the crossbreed of Rhode Island Red and a synthetic brown layer Freire et al. (2008) had used. In February, we used 26 male crossbred chicks of the broiler strain 'Australorps' and a layer-strain 'White Leghorn' (Barter and Sons Hatchery, Luddenham, NSW, Australia), similar to the white broilers used by Freire et al. (2008). The chicks were delivered to the laboratory on the day after hatching (day 2), randomly divided into four groups of 10 -12 (January) and 5 -7 (February) chicks and moved to their housing boxes made from cardboard (94 x 44.5 x 63 cm). The floor was covered with 5 cm of wood shavings. A drinker (10 cm Ø) in the middle of the box was always present.

Training and preparing for the tests

The total intensity of the local geomagnetic field at the test site was 58 µT. Two coils of copper wire (14 cm Ø) with 60 windings each were placed underneath the housing box so that the chicks could not detect them by pecking or scratching the floor. One coil, the *magnetic coil*, had the windings wrapped in just one direction; when activated by a 1 A current, it produced a magnetic anomaly with a maximum of 345 µT, about 6 times the intensity of the local geomagnetic field, and an inclination downward instead of upward. It was positioned near the middle of one shorter side of the box, and the food bowl was placed above it (see below). The other coil, the *control coil*, was double-wrapped with 30 windings in one direction and 30 windings in the opposite direction, so that when activated, it did not induce a net magnetic field. It was placed near the opposite shorter side of the box. No food bowl was placed on top of this coil to avoid an association of no anomaly and food. The position of the coils was changed in a random sequence.

At the age of 2 days, we began training the chicks so that they could form an association between the magnetic anomaly and food. Both coils were activated and after three minutes, the food bowl was placed above the magnetic coil in the housing box, where it was left for about 10-20 min, depending on when feeding stopped. After that, the food bowl was removed and the coils were switched off. This procedure was repeated six times a day until day 9 after hatching, the first day of testing. From this day on, it was repeated five times a day.

Critical tests

The testing arena was located in another room. The square arena (80 x 80 cm) had white sides and lighting from four incandescent bulbs above each corner, designed to minimise external directional cues. It was similar to the arena used by Freire et al. (2005), but without the small screens in each corner. The coils were placed underneath the chipboard floor, where they did not produce any measurable heat when activated. No food bowl or food was present in the testing arena.

From day 5 onward, the chicks were placed in the testing arena to familiarise them with the arena and the treatment. Since chicks at this young age are frightened when alone, they were released into the arena in pairs chosen arbitrarily from the same box. They remained there for about 5 min, after which they were returned to their housing box.

The testing procedure was similar to the one during the previously described familiarising procedure. Two coils like the ones described above were placed underneath the floor of the arena in two opposite corners, one producing a magnetic anomaly, the control coil without producing a net field (Fig. 1). The test apparatus was rotated in a random sequence at the end of each day, and the position of the coils was varied between days to prevent the chicks from using other cues within or outside the arena.

From day 9 to day 20, we selected 7 chicks in January and 5 chicks in February for testing each day. They were tested in the morning prior to first feeding to ensure that feeding motivation was at its highest. The two chicks were placed in the centre of the arena and remained there for 5 minutes once per test-day. One chick was marked with a coloured dot on the head, and only this one was scored. A second chick, again chosen arbitrarily from the same box, was added as by-runner, but was not scored. A chicken could be an experimental bird in one session and act as by-runner in another. Chicks were tested up to three times.

The chicks' behaviour in the arena was recorded by videotape for later analysis. The monitor was equipped with a diaphanous foil to divide the floor of the arena into 16 small subsquares of equal size. For the analysis (see below), we combined four small subsquares, resulting in four quadrants, one for each corner, which were numbered from A to D. The one with the magnetic coil was always quadrant

A; the opposite one with the control coil was D (see Fig. 1). During the 5 min duration of each test, the location of the marked chick was scored every 10 seconds, resulting in 31 counts per test, with their distribution proportional to the time the chick spent in each of the four quadrants.

The chicks had a chance to find the quadrant with the magnetic anomaly only when moving around in the box (see Fig. 1). Hence we considered only periods during which the chicken were active searching around. The brown chicks were always active and scored 31 times per test; the White Leghorn X Australorps, however, were generally less active and 5 chicks never scored because they were always inactive. Others spent extended periods not moving. Here we applied the following criteria: (1) Tests where the chick stayed for 20 or more counts in just one of the 16 small subsquares without moving were excluded from the analysis. (2) If a chick stayed for 10 consecutive counts in the same small subsquare, then just this particular sequence was excluded, and the remaining counts analysed.

Data analysis

The data analysis was based on the video recordings. The videotapes were analysed by one person (D.K.) in a single-blind fashion.

Because not all chicks were active, the counts indicating the number of times the chick was observed in any of the four quadrants vary between 12 and 31. If a chick was tested more than once, we calculated the mean. To test if the scores were significantly different from chance, we used a one-sided t-test, and find out whether quadrant A is preferred over the others we used the t-test for joint samples.

Results

Figure 2 summarizes the mean percentage of the counts in the four quadrants over the entire testing period. Table 1 gives these data numerically, together with the sum of all mean counts in the four quadrants in all tests. (For an analysis based on the raw data, see Electronic Supplemental Material).

In brown as well as in white chicks, the number of counts were significantly higher than chance in quadrant A, and significantly lower than chance in quadrant D, quadrant B and C were intermediate and did not differ from chance (see Fig. 2). The brown chicks spent significantly more time in the quadrant A with the magnetic anomaly than in the other three quadrants (A to B: $p = 0.027$; A to C: $p = 0.042$; A to D: $p < 0.01$); for the white chicks, there is a significant difference between the time spent in quadrants A and D ($p < 0.001$), while the difference between A and B or C does not reach significance ($p = 0.051$ and $p = 0.052$, respectively).

Discussion

In brown as well as in white chicks, the number of counts is highest in quadrant A, indicating a preference for this quadrant with the magnetic anomaly. The overall distribution of counts, with the lowest number in the opposite quadrant D and B and C intermediate, reflects that the chicks had to move around to discover the anomaly. Our findings are the first to show that young chicks can sense changes in the magnetic field and are able to associate a magnetic anomaly with food; the two previous successful attempts to train birds to respond to a magnetic anomaly had both used homing pigeons (Mora et al. 2004; Thalau et al. 2007).

We started our critical tests on day 9 after hatching. Chicks placed into the arena earlier to familiarise themselves with the test arrangements appeared rather fearful and barely moved. Their behaviour changed markedly from day 9 onward, when they became more active and began to explore their surroundings. This change in behaviour correlates with the maturation of several cognitive brain functions observed during this period, such as the lateralisation of food searching and use of distant visual cues to discriminate between food and non-food items (see Rogers 1995 for review). Active movements were essential for our test design: the magnetic anomaly, which served as a marker, was produced by a small coil underneath one corner; therefore, it was restricted to a limited part of the arena. The chicks were released in the centre where the magnetic field was still unchanged, thus providing no clue in which corner the anomaly was presented – they could detect its presence only by moving around and entering the area where the magnetic field was altered. Recent studies with homing pigeons (Schiffner et al. 2011, Schiffner and Wiltschko 2011) suggest a sensitivity for differences in intensity in the range of 10 – 20 nT, but possibly the chicks need a somewhat greater difference to sense this.

When the chicks reached the quadrant with the anomaly, they often raised their heads and looked upward as they did in their housing boxes just before feeding. This behaviour was not observed in the other quadrants. It recalls the "head scans" described by Mouritsen et al. (2004), although our chicks moved their head in the vertical rather than the horizontal plane.

The stimulus itself consisted of a very rapid change in magnetic intensity within a radius of about 20 cm, accompanied by a reversal in inclination above the centre of the coil. Inclination reversals are changes in the spatial direction of the magnetic field and are probably detected by the cryptochrome system in the eye (Ritz et al. 2004, 2009; Thalau et al 2005), while the intensity changes are probably monitored by the magnetite-based receptors in the beak (Semm and Beason 1990; Fleissner et al. 2003; Mora et al. 2004; Falkenberg et al. 2010; Wiltschko et al. 2010). Our experiments do not allow us to distinguish which of these changes was crucial for the chicks to recognise the anomaly, and we can therefore not decide which of the two systems was involved. In previous experiments, a brown layer strain, closely related to the one used here, had been able to detect magnetic compass directions (Freire et al. 2005, 2008) by radical pair processes (Wiltschko et al. 2007), obviously using the same mechanisms as European robins, *Erithacus rubecula* (Turdidae). A white strain, also closely related to the white chicks used here, could not be trained to magnetic directions. In the present study, however, the white chicks also clearly preferred the quadrant with the anomaly. If they were also unable to detect magnetic directions, they could have detected the change in magnetic intensity with the help of the magnetite-based receptors in the upper beak, as studies with homing pigeons suggest (Mora et al. 2004; Wiltschko et al. 2010; Schiffner et al. 2011). The brown layers could also have used these receptors to detect the change in intensity, but they might have additionally detected the change in inclination, i.e. the direction of the magnetic field, with the help of the radical pair mechanism.

Acknowledgment

This work was supported by the Deutsche Forschungsgemeinschaft (grants to W.W.). We thank Gary McKenzie for assistance with the methodology, Brooks Ferebee and Ingo Schiffner for statistical advice. The experiments were performed in accordance with the rules and regulations of Animal Welfare in Australia.

Literature

Beason RC, Semm P (1996): Does the avian ophthalmic nerve carry magnetic navigational information? J Exp. Biol 199:1241–1244

Falkenberg G, Fleissner G, Schuchardt K, Kuehbacher M, Thalau P, Mouritsen H, Heyers D, Wellenreuther G, Fleissner G (2010) Avian magnetoreception: elaborate iron mineral containing dendrites in the upper beak seem to be a common feature of birds. PLoS ONE 5:e9231

Fleissner G, Holtkamp-Rötzler E, Hanzlik M, Winklhofer M, Fleissner G, Petersen N, Wiltschko W (2003) Ultrastructural analysis of a putative magnetoreceptor in the beak of homing pigeons. J Comp Neurol 458:350–360

Freire R, Birch T (2010) Conditioning to magnetic direction in the Pekin duck (*Anas platyrhynchos domestica*). J Exp Biol 213:3423-3426

Freire R, Munro U, Rogers LJ, Sagasser S, Wiltschko R, Wiltschko W (2008) Different responses in two strains of chicken (*Gallus gallus*) in a magnetic orientation test. Anim Cogn 11:547-552

Freire R, Munro UH, Rogers LJ, Wiltschko R, Wiltschko W (2005) Chicken orient using a magnetic compass. Curr Biol 15:R620-621

Heyers D, Zapka M, Hoffmeister M, Wild JM, Mouritsen H (2010) Magnetic field changes activate the trigeminal brainstem complex in a migratory bird. Proc Natl Acad Sci USA 107:9394-9399

Keary N, Ruploh T, Voss J, Thalau P, Wiltschko R, Wiltschko W, Bischof HJ (2009) Oscillating magnetic field disrupts magnetic orientation in Zebra finches, *Taeniopygia guttata.* Front Zool 6:25

Mora C, Davison M, Wild JM, Walker MM (2004) Magnetoreception and its trigeminal mediation in the homing pigeon. Nature 432:508-511

Mouritsen H, Feenders G, Liedvogel M, Kropp W (2004) Migratory birds use head scans to detect the direction of the earth’s magnetic field. Curr Biol 14:1946–1949

Muheim R, Edgar NM, Sloan KA, Phillips JB (2007) Magnetic compass orientation in C57BL/6J mice. Learn Behav 34:366-373

Ritz T, Thalau P, Phillips JB, Wiltschko R, Wiltschko W (2004) Resonance effects indicate a radical-pair mechanism for avian magnetic compass. Nature 429:177-180

Ritz T, Wiltschko R, Hore PJ, Rodgers CT, Stapput K, Thalau P. Timmel CR, Wiltschko W (2009) Magnetic compass of birds is based on a molecule with optimal directional sensitivity. Biophys J 96:3451-3457

Rogers L (1995) The Development of Brain and Behaviour in the Chicken. CAB International, Wallingford.

Rogers L, Munro U, Freire R, Wiltschko R, Wiltschko W (2008) Lateralized response of chicks to magnetic cues. Behav Brain Res 186:66-71

Salzen, EA, Lily RE, McKeown JR (1971) Colour preference and the imprinting in domestic chicks. Anim Behav 19:542-547

Semm P, Beason RC (1990) Responses to Small Magnetic Variations by the Trigeminal System of the Bobolink. Brain Res Bull 25:735-740

Schiffner I, Wiltschko R (2011) Temporal fluctuation of the geomagnetic field affect pigeons' entire homing flight. J Comp Physiol A 197:765-772

Schiffner I, Fuhrmann P, Wiltschko R (2011) Tracking pigeons in a magnetic anomaly and in magnetically 'quiet' terrain. Naturwissenschaften 98:575-581

Shcherbakov D, Winklhofer M, Petersen N, Steidle J, Hilbig R, Blum M (2005) Magneto-sensation in zebrafish. Curr Biol 15:R161-162

Thalau P, Ritz T, Stapput K, Wiltschko R, Wiltschko W (2005) Magnetic compass orientation of migratory birds in the presence of a 1.315 MHz oscillating field. Naturwissenschaften 92:86-90

Thalau P, Holtkamp-Rötzler E, Fleissner G, Wiltschko W (2007) Magnetic conditioning to locate food in homing pigeons, *Columba livia* f. *domestica*. Naturwissenschaften 94:813-819

Vallortigara G, Andrew RJ (1994) Olfactory lateralization in the chick. Neuropsychologia 32:417-423

Vallortigara G, Regolin L, Rigoni M, Zanforlin M (1998) Delayed search for a concealed imprinted object in the domestic chick. Anim Cogn 1:17-24

Voss J, Keary N, Bischoff HJ (2007) The use of the geomagnetic field for short distance orientation in zebra finches. Neuroreport 18:1053–1067

Wiltschko R, Wiltschko W (1995) Magnetic Orientation in Animals. Springer, Berlin, Heidelberg, New York

Wiltschko R, Schiffner I, Fuhrmann P, Wiltschko W (2010) The role of the magnetite-based receptors in the beak in pigeon homing. Curr Biol 20:1534-1538

Wiltschko W, Wiltschko R (2007) Magnetoreception in birds: two receptors for two different tasks. J Ornithol 148 (Suppl 1):S61-S76.

Wiltschko W, Freire R, Munro U, Ritz T, Rogers L, Thalau P, Wiltschko R (2007) The magnetic compass of domestic chickens, *Gallus gallu*s. J Exp Biol 210:2300-2310

Wilzeck C, Wiltschko W, Güntürkün O, Buschmann JU, Wiltschko R, Prior H (2010) Learning of magnetic compass directions in pigeons. Anim Cogn 13:443–451

Zapka M, Heyer D, Hein CM, Engels S, Schneider N-L, Hans J, Weiler S, Dreyer D, Kishkinev D, Wild JM, Mouritsen H (2009) Visual but not trigeminal mediation of magnetic compass orientation in a migratory bird. Nature 461:1274-1278

Table 1 Distribution of the mean number of counts in the four quadrants, together with the percentage for both strains of chicken. N, number of chicks; n_{tot}, total number of counts. $P_{t\text{-test}}$, p of the t-test, with asterisks indicating significance.

N		n_{tot}	**Quadrant**			
			A	**B**	**C**	**D**
Brown Layer chicks						
44	**counts**	**1364**	**423**	**319**	**331**	**291**
	percent		**31%**	**23%**	**24%**	**21%**
	$P_{t\text{-test}}$		**0.008****	**0.251**	**0.372**	**0.05***
White Leghorn X Australops chicks						
21	**counts**	**596**	**222**	**153**	**139**	**83**
	percent		**37%**	**26%**	**23%**	**14%**
	$P_{t\text{-test}}$		**0.005****	**0.337**	**0.410**	**< 0.001*****

Fig. 1: Changes in magnetic intensity induced by the magnetic coil placed under the corner of one subsquare. Intensity was measured above the activated coil in steps of 5 cm from its centre. The white area with intensity below 58.0 µT indicates the unchanged geomagnetic field.

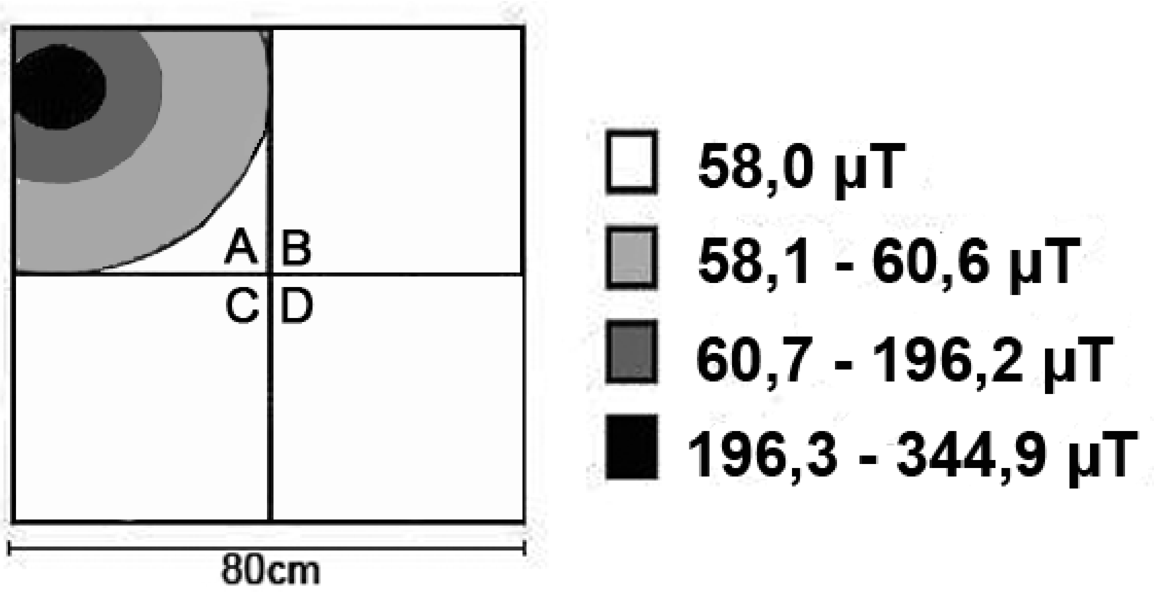
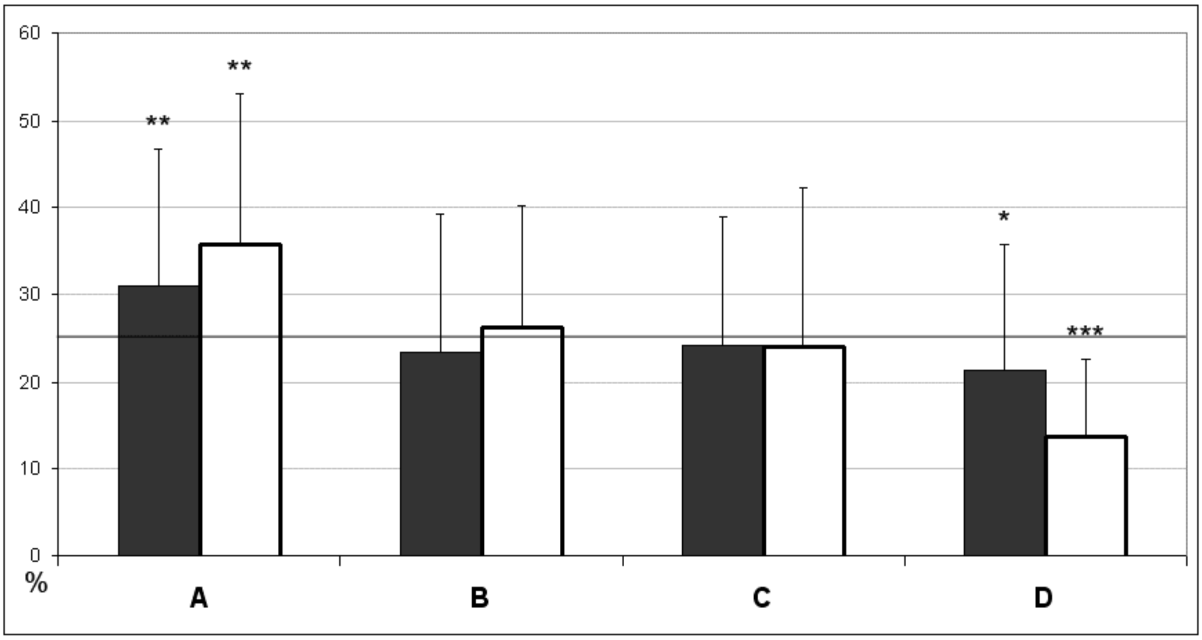


Fig. 2: Mean number of choices (in percent) in the four quadrants A, B, C and D, with standard deviation indicated. The data from the Brown Layer are given in black, those from the White Leghorn X Australorps in grey. The chance level of 25 % is marked by a black line; significant differences from chance are indicated by asterisks (t-test): * < 0.05, ** < 0.01, *** < 0.001.



Electronic Supplementary Material

Conditioning domestic chickens to a magnetic anomaly

Susanne Denzau[1], Dany Kuriakose[1], Rafael Freire[2], Ursula Munro[3], Wolfgang Wiltschko[1]

[1] FB Biowissenschaften, J. W. Goethe-Universität Frankfurt, Germany

[2] School of Animal and Veterinary Science, Charles Sturt University, Wagga Wagga, Australia

[3] The School of the Environment, University of Technology, Sydney, Australia

Table S1: Performance of the individual brown layer chicks in the single tests. In the chick number, the first digit refers to the housing box.

			Subsquare			
Chick number	testing day	total	A	B	C	D
1.1	day 13	31	15	3	5	8
1.2	day 17	31	3	1	3	24
1.3	day 09	31	3	6	7	15
1.4	day 09	31	14	10	3	4
1.4	day 13	31	12	6	8	5
1.5	day 09	31	7	24	0	0
1.5	day 13	31	16	0	15	0
1.5	day 17	31	11	10	4	6
1.6	day 09	31	12	1	10	8
1.6	day 13	31	17	3	8	3
1.6	day 17	31	12	14	0	5
1.7	day 13	31	0	0	4	27
1.7	day 17	31	15	6	9	1
1.8	day 09	31	5	5	16	5
1.8	day 13	31	19	5	5	2
1.8	day 17	31	9	18	2	2
1.12	day 09	31	14	4	7	6
1.12	day 13	31	23	3	1	4
1.12	day 17	31	5	8	9	9
1.14	day 09	31	0	12	0	19
1.14	day 13	31	9	0	14	8
1.14	day 17	31	28	1	2	0
2.1	day 12	31	19	3	6	3
2.1	day 16	31	13	1	12	5
2.1	day 20	31	12	6	10	3
2.2	day 12	31	13	0	18	0
2.2	day 16	31	6	12	4	9
2.2	day 20	31	2	5	11	13
2.3	day 12	31	1	26	0	4
2.3	day 16	31	7	18	5	1
2.3	day 20	31	4	10	9	8
2.6	day 12	31	2	16	10	3
2.6	day 16	31	21	2	4	4
2.6	day 20	31	8	10	7	6
2.7	day 12	31	20	3	7	1
2.8	day 12	31	10	2	3	16
2.8	day 16	31	0	0	29	2
2.9	day 16	31	13	2	12	4
2.9	day 20	31	7	9	6	9

2.10	day 12	31	5	1	12	13
2.10	day 20	31	21	0	4	6
2.11	day 12	31	4	3	3	21
2.11	day 16	31	9	8	3	11
2.13	day 16	31	11	6	9	5
2.13	day 20	31	0	7	18	6
2.15	day 20	31	1	9	11	10
3.1	day 11	31	3	5	12	11
3.1	day 15	31	1	21	9	0
3.3	day 11	31	11	6	4	10
3.3	day 15	31	19	4	3	5
3.3	day 19	31	11	5	8	7
3.4	day 19	31	4	7	6	14
3.5	day 11	31	16	5	3	7
3.5	day 15	31	17	12	1	1
3.6	day 11	31	7	3	9	12
3.6	day 19	31	11	6	10	4
3.7	day 11	31	13	4	7	7
3.9	day 11	31	8	23	0	0
3.9	day 15	31	18	7	1	5
3.9	day 19	31	4	11	9	7
3.10	day 15	31	10	8	8	5
3.10	day 19	31	10	14	3	4
3.12	day 11	31	6	8	11	6
3.12	day 15	31	13	11	3	4
3.12	day 19	31	12	7	3	9
3.13	day 15	31	19	12	0	0
3.13	day 19	31	9	11	3	8
3.15	day 11	31	9	7	8	7
3.15	day 15	31	13	9	3	6
3.15	day 19	31	9	7	10	5
4.1	day 14	31	21	2	7	1
4.1	day 18	31	3	7	5	16
4.2	day 10	31	5	11	8	7
4.2	day 14	31	8	3	8	12
4.2	day 18	31	11	7	11	2
4.3	day 18	31	8	9	7	7
4.4	day 10	31	3	6	20	2
4.4	day 14	31	6	5	8	12
4.4	day 18	31	30	1	0	0
4.5	day 10	31	2	0	29	0
4.5	day 14	31	11	12	1	7
4.5	day 18	31	6	9	10	6
4.6	day 14	31	0	0	31	0
4.6	day 18	31	5	18	3	5
4.7	day 10	31	3	1	26	1
4.8	day 10	31	14	7	6	4
4.9	day 18	31	1	29	0	1
4.10	day 10	31	9	12	2	8
4.10	day 14	31	12	7	8	4
4.12	day 10	31	15	6	9	1
4.12	day 14	31	14	5	10	2
4.12	day 18	31	1	13	5	12
4.15	day 10	31	23	0	7	1
4.15	day 14	31	19	10	1	1

Table S2: Performance of the individual white broiler chicks in the individual tests. In the chick number, the first digit refers to the housing box.

chicken	testing day	total	A	B	C	D
5.1	day 09	31	9	10	1	11
5.1	day 13	31	9	11	4	7
5.2	day 09	20	4	0	8	8
5.2	day 13	31	10	16	2	3
5.2	day 17	31	9	12	3	7
5.5	day 09	31	9	8	4	10
5.5	day 13	31	14	8	4	5
5.5	day 17	12	0	9	0	3
5.7	day 13	31	14	6	9	2
5.9	day 13	31	3	0	23	5
6.2	day 12	31	8	20	0	3
6.2	day 20	31	13	5	7	6
6.3	day 12	19	3	1	14	1
6.3	day 16	31	18	2	3	8
6.3	day 20	31	5	22	2	2
6.5	day 12	31	17	4	3	7
6.5	day 16	31	10	9	5	7
6.5	day 20	31	21	3	7	0
6.6	day 16	31	11	10	6	4
6.6	day 20	31	12	8	10	1
6.7	day 12	31	12	6	8	5
6.7	day 16	31	16	12	2	1
6.7	day 20	31	10	11	0	10
7.1	day 19	31	7	8	10	6
7.3	day 15	13	0	7	5	1
7.8	day 19	31	14	2	13	2
7.10	day 15	19	5	0	12	2
7.10	day 19	31	0	14	8	9
8.1	day 11	31	14	3	14	0
8.2	day 14	31	16	7	6	2
8.4	day 10	31	17	14	0	0
8.5	day 18	31	23	1	0	7
8.7	day 10	16	1	9	0	6
8.7	day 14	18	11	3	2	2
8.7	day 18	31	0	3	20	8
8.8	day 10	31	0	21	0	10
8.8	day 18	31	28	2	1	0
8.10	day 10	31	9	17	5	0
8.10	day 11	15	10	0	5	0

The distribution of the raw data in the four quadrants is given in Figure S1. For their analysis, we used a binominal Generalised Linear Mixed Model (GLMM, Genstat 13th Edition, VSN International) and the Permutation test (Good 2005), two tests designed for handling inhomogenious material with multiple measurements. With the Generalised Linear Mixed Model, our variable was the counts in each quadrant, with the total counts being the sum of scores of the chicks in all four quadrants. Quadrant (A, B, C, and D) and age (day of testing) were included as factors in the model and chick as a random effect. Since the chicks were kept in different home pens, home pen was initially included in the model, but since it had no effect, it was subsequently removed.

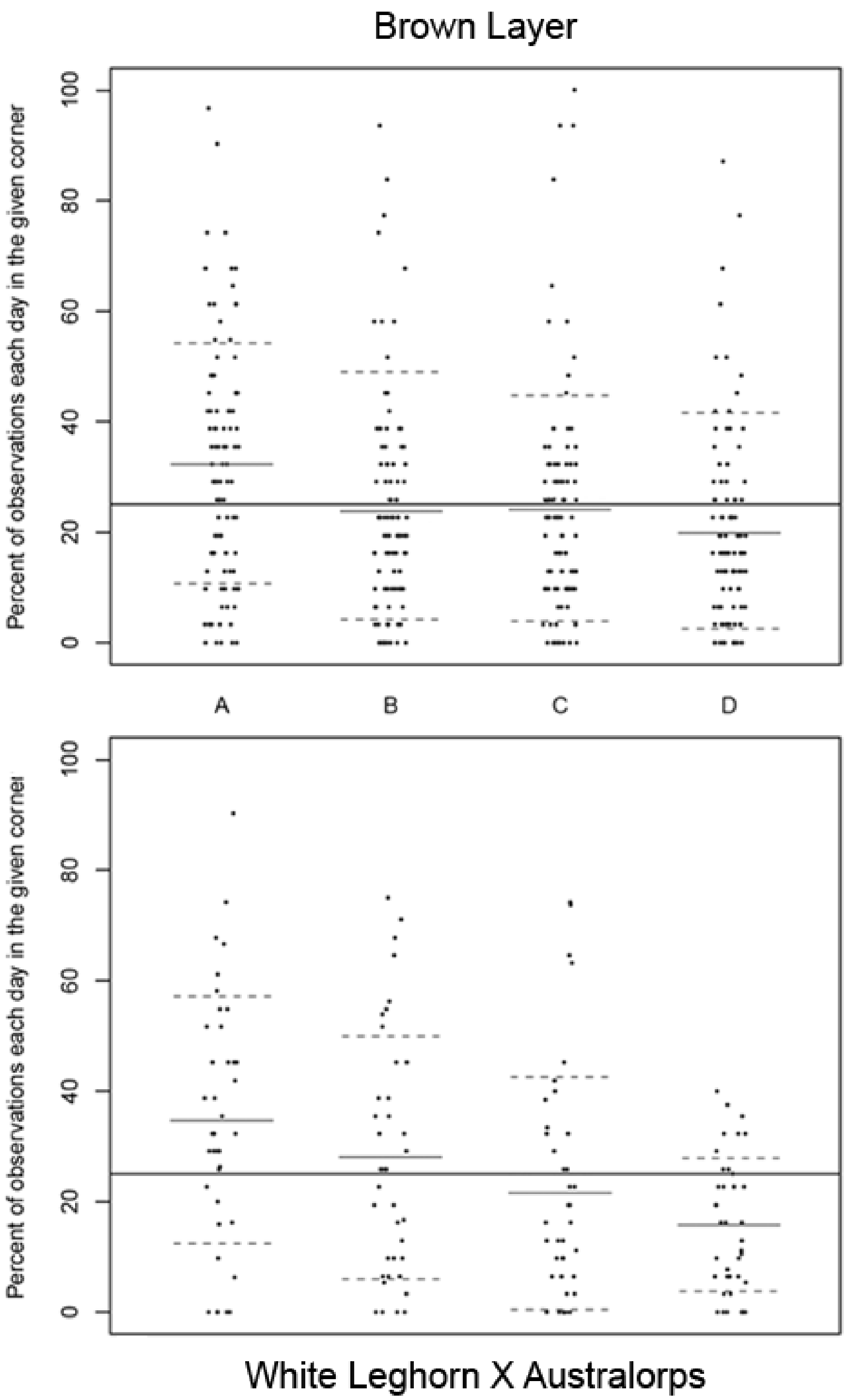
For both strains, there were no significant main effects of day (GLMM, $F_{11,328}$=0.04, P=1 and $F_{11,108}$= 0.19, P = 0.99) or corner/day interaction (GLMM, $F_{33,328}$=1.47, P=0.051 and $F_{33,108}$ =1.24, P = 0.20). A significant difference is shown when choosing the four corners (see Table S3).

The permutation test with 10 000 repetitions was used to check whether the choices in the four quadrants deviated significantly from chance. The results are given in Table S3. The distribution of counts in the four quadrants is not equal. Chicks of both strains preferred the quadrant with the magnetic coil above chance level.

Table S3: Distribution of the number of counts in the four quadrants (all tests summarised) together with the percentage for all counts of both strains of chicken. n_{tot}: total counts P_{perm}, probability for deviation from random by the Permutation test.

			Quadrant					
N		**n_{tot}**	**A**	**B**	**C**	**D**	**GLMM**	
Brown Layer chicks								
44	**counts**	**2914**	**941**	**692**	**701**	**580**	**$F_{3,328}$ = 5.8**	**P < 0.001**
	percent		**32%**	**24%**	**24%**	**20%**		
	P_{perm}		**< 0.001**	**0.29**	**0.34**	**< 0.01**		
White Leghorn X Australorps chickens								
21	**counts**	**1093**	**392**	**304**	**226**	**171**	**$F_{3,108}$ = 6.6**	**P < 0.01**
	percent		**36%**	**28%**	**21%**	**16%**		
	P_{perm}		**< 0.01**	**0.18**	**0.15**	**< 0.001**		

Fig. S1: Choices in the four quadrant (in percent) of all chicks during the entire testing period. The chance level of 25 % is marked by a black line, the mean is marked with a grey dash,with dotted lines showing the standard deviation.



Brown Layer

White Leghorn X Australorps

Literature:

Good PI (2005): Permutation, Parametric, and Bootstrap Tests of Hypotheses. Springer, New York.