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Report

The Role of the Magnetite-Based Receptors in the Beak in Pigeon Homing

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Summary

Magnetite-containing structures in the upper beak of birds have been described as putative magnetoreceptors [1–4], but so far, all positive evidence indicating their influence on behavior has come from laboratory studies using rather unnatural stimuli (e.g., [5–7]). Here, we demonstrate these receptors' possible role in a natural situation: we released pigeons with these receptors deactivated by a local anesthetic within and outside a magnetic anomaly, together with untreated control birds. Within the anomaly, the untreated birds showed unusually long vanishing intervals and scattered bearings, indicating confusion by the anomalous magnetic conditions. Anesthesia of the beak suppressed this adverse effect. Outside the anomaly, in contrast, the treatment had little effect. These findings indicate that the receptors in the beak mediate magnetic "map" information and that this information is normally included in the navigational process yet can be replaced by nonmagnetic factors at most sites.

Results and Discussion

About 10 years ago, clusters of superparamagnetic magnetite in the beak of homing pigeons, *Columba livia* f. *domestica*, were suggested to be part of a magnetoreception system [1–3]. Meanwhile, similar structures have also been found in other bird species [8]. Theoretically, these putative magnetoreceptors could mediate information on direction as well as on magnetic intensity (e.g., [9–11]); electrophysiological recordings suggest that they are intensity receptors [12]. Behavioral studies producing evidence for their effect on behavior [5–7], however, have involved typical laboratory situations with stimuli that do not occur in nature. In view of this, it seemed necessary to demonstrate the behavioral relevance of these receptors under natural conditions and to identify their normal function.

Studies with homing pigeons had revealed marked effects of the conditions found in strong magnetic anomalies [13–15]: the bearings show increased scatter leading up to disorientation, with shorter vectors and prolonged vanishing intervals. These findings offered an opportunity to test the behavioral relevance of the receptors in the upper beak: if they mediate changes in magnetic intensity, then deactivating them with a local anesthetic should suppress the effect of the anomalous magnetic conditions.

To test this hypothesis, we performed releases at four sites within the Vogelsberg anomaly about 60 km northeast of our Frankfurt loft (50°08′N, 8°40′E) and compared the pigeons' behavior here with that observed at four sites in magnetically "quiet" terrain. The positions of the release sites, together with an overview of the magnetic conditions in the Frankfurt region, are given in Figure 1; for details on the magnetic conditions at the test sites, see Figure 2 and Supplemental Information Part 1 available online. Supplemental Information Part 2 discusses the nature of the magnetic "map" factors.

The data of the individual releases are given in Supplemental Information Part 3. An interesting pattern emerges: in all cases of significant differences within the anomaly, the experimental birds with their beaks anesthetized had longer vectors and shorter vanishing intervals, whereas in magnetically quiet terrain, this trend was reversed. We found a consistent difference in initial orientation between experimental and control birds only at the control site C4, Bickenbach ($F_{2,3} = 27.19$, $p < 0.05$, Hotelling's two-sample test for bivariate samples).

Figure 3 gives the means (± standard deviation) of the absolute deviation from the home direction (Figure 3A), vector lengths (Figure 3B), and median vanishing intervals (Figure 3C) for controls and experimentals within and outside the anomaly; significant differences between groups by two-way analysis of variance (ANOVA) are indicated. The absolute deviations from the home direction show substantial scatter, with no significant differences between groups (Figure 3A). For vector length (Figure 3B), the ANOVA shows a significant interaction ($p = 0.027$). The mean vector lengths of the controls released within the anomaly were significantly shorter than those of the controls outside the anomaly, whereas those of the experimentals were similar, and also similar to those of the control birds at the control sites.

The greatest differences were found in the vanishing intervals (Figure 3C). A significant interaction ($p = 0.0002$) indicates a change in the relationship between controls and experimentals within and outside the anomaly. Within the anomaly, the vanishing intervals of the controls were significantly longer than those of the experimentals, whereas there was no difference between the two groups outside. At the same time, the vanishing intervals of the controls within the anomaly were significantly longer than outside, whereas there was no such difference found in the experimentals—that is, the controls within the anomaly were the slowest to vanish from sight.

In summary, within the anomaly we find a trend that temporarily disabling the receptors in the upper beak by anesthesia speeds up the birds' departure and tends to improve orientation, whereas it seem to have no effect or a slightly adverse effect in magnetically quiet regions. This trend is primarily the result of the behavior of the control birds, which are negatively affected by the magnetic conditions within the anomaly.

Effects of Deprivation of Magnetic "Map" Information within and outside the Anomaly

Deviations from the home direction were highly variable; we did not find a consistent difference between controls and experimentals. Within the anomaly itself, this is not surprising: we cannot expect an effect of anesthesia there, because both groups have to rely on the same nonmagnetic factors. The experimentals use these factors because they are the only

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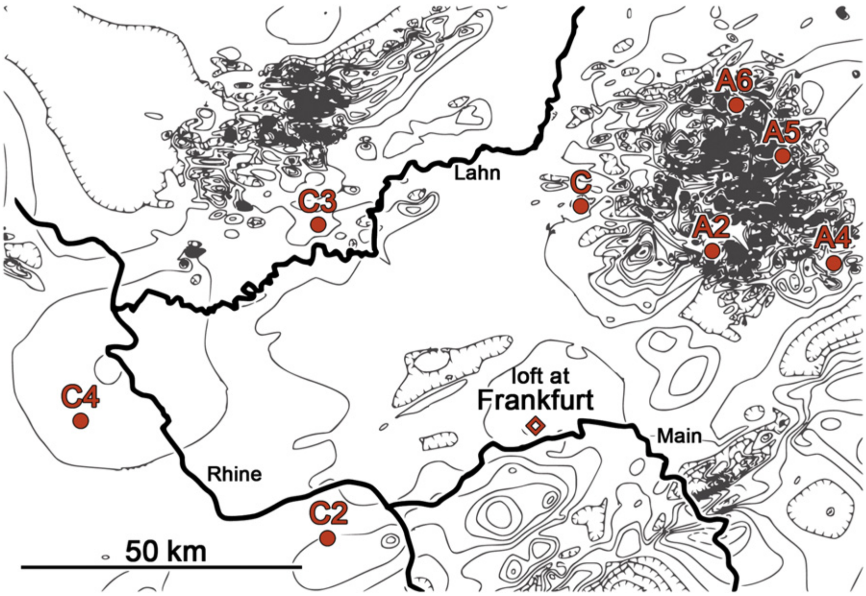

Figure 1. Magnetic Conditions in the Frankfurt Region and the Position of the Release Sites

The isolines, 25 nanoteslas apart, indicate the differences to the magnetic reference field, i.e., from the values expected if the magnetic field were regular; isolines with tick marks indicate negative deviations. In the Vogelsberg anomaly northeast of Frankfurt and in an area to the northwest, the field changes very quickly, leading to isolines so close that they can no longer be separated. The loft at Frankfurt am Main is marked by an open diamond; the positions of the eight release sites are indicated by red dots. Sites used in the previous study [15] have the same number.

cues available to them, and the controls, unable to interpret the rapidly changing field within the anomaly, finally recognize its unreliability and also have to turn to nonmagnetic cues. If magnetic factors played a major role, we would expect to find differences at the control sites. This may be the case at site C4 (see below).

We found differences mainly in vector length and vanishing intervals, the two variables that, in an earlier study [15], had been found to be correlated with the local magnetic conditions. In both cases, the control data obtained within the anomaly and those from outside differed significantly: the highly irregular magnetic field within the anomaly leads to significantly shorter mean vectors and significantly longer vanishing intervals. The rapidly changing steep gradients obviously lead to confusion and thus delay the birds' departure. When the pigeons finally decide to take off after flying around erratically, they are located in various directions at considerable distances from the release point, which causes them to be lost from sight with considerable scatter, resulting in shorter vectors. Temporarily depriving pigeons of this confusing magnetic input by local anesthesia leads to longer vectors and shortens the vanishing intervals. The effect on vanishing intervals is highly significant—it represents the most

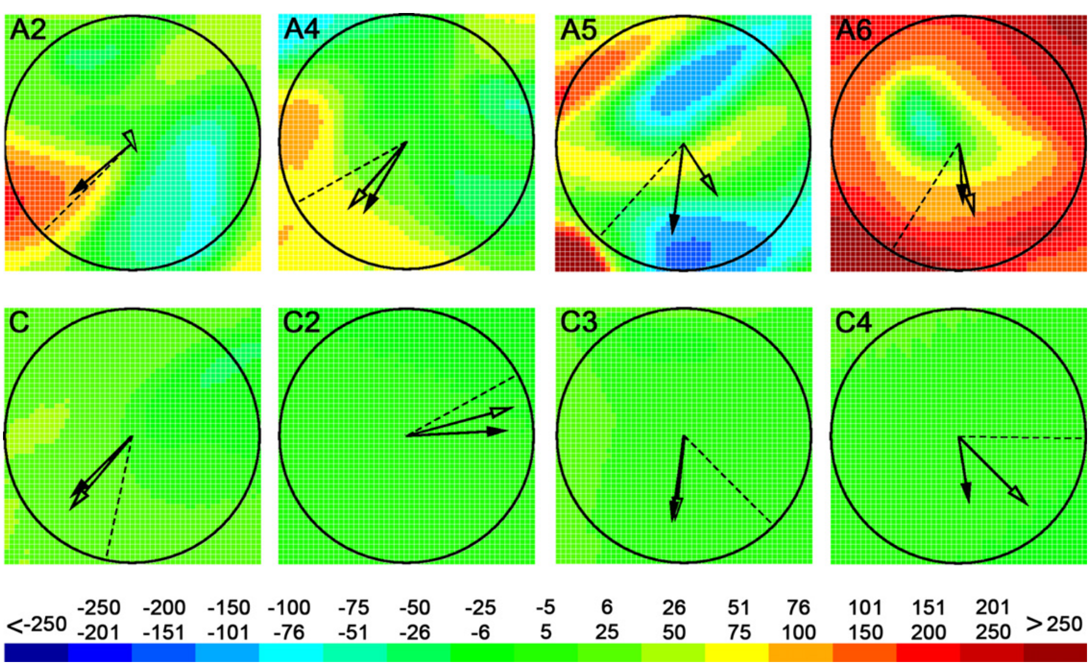

Figure 2. Magnetic Conditions at the Release Sites

The differences between the local magnetic intensity and the intensity at the release point are given in nanoteslas. Yellow and red indicate increasing intensities; blue indicates decreasing intensities (note that the scale is not linear). The circles have a radius of 2.5 km; the dashed radius indicates the home direction. The two arrows represent the mean vectors based on the bearings of all three releases per site: open arrowhead, untreated control birds; solid arrowhead, experimental pigeons deprived of magnetic information by local anesthesia of the upper beak.

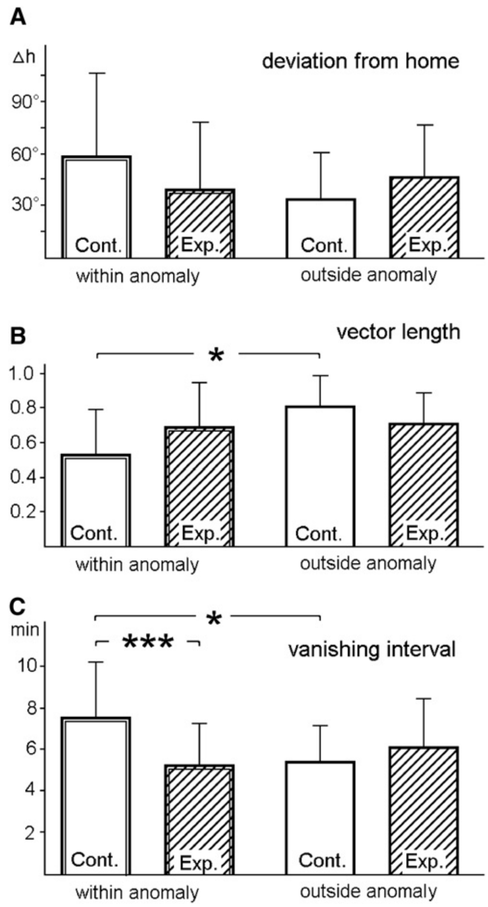

Figure 3. Means and Standard Deviations of the Variables Recorded in the Individual Releases

White columns indicate untreated control pigeons (Cont.); shaded columns indicate experimental pigeons (Exp.) whose upper beaks were locally anesthetized to deprive them of magnetic "map" information. Columns with double borders indicate data from within the anomaly area; columns with a single border indicate data from outside the anomaly area. *$p < 0.05$, ***$p < 0.001$ between groups by two-way analysis of variance.

direct evidence of an involvement of the receptors in the upper beak in the navigational process.

Local anesthesia of the beak seems to have little effect outside the magnetic anomaly. In a few cases, it led to shorter vectors and longer vanishing intervals, but an overall trend did not become obvious. This means that neither a possible nonspecific effect of the treatment nor the unavailability of magnetic "map" factors represents a severe interference—pigeons can cope with both. The observation that about half of the significant differences were found at site C4, Bickenbach (see Supplemental Information Part 3), where we also found the only significant difference in initial orientation between experimental and control birds, seems to suggest that magnetic factors at this site are helpful and more important than at the other control sites. Interestingly, treating pigeons with a magnetic pulse had also produced a noticeable effect at this site in a previous study [16].

The Role of Magnetic "Map" Factors in Avian Navigation

The behavior of the untreated control birds indicates that magnetic information is part of the normal navigational processes: when available, magnetic "map" information seems to be regularly consulted. It is, however, just one factor among several others as indicated by the behavior of the birds deprived of this input. The magnetic conditions within the anomaly are so irregular that our birds could not have found their way out of the anomaly if they had to rely on magnetic factors alone—except, perhaps, by flying straight in random directions until they had left the area with the anomalous field.

A multimodal navigational "map" including factors of different nature helps to explain some of the seemingly conflicting results found in the literature. Releasing pigeons in the strong Iron Mine anomaly in the northeastern United States, Walcott [13] observed disoriented behavior, possibly suggesting the "random flying" strategy mentioned above; released in the same anomaly a second time, the birds were oriented [17]. Releases in the Kaiserstuhl anomaly in southwestern Germany confirmed these findings [14]. In the Vogelsberg anomaly, although it is similarly rugged with steep, irregular gradients, untreated pigeons are not always disoriented, occasionally even with long vectors ([15] and present study). This does not argue against the use of magnetic "map" factors—rather, it appears to reflect the availability and reliability of nonmagnetic factors. In the Vogelsberg anomaly, such factors seem to be readily available, as suggested by the vanishing intervals of the experimental birds being in the same range as those of the controls birds outside the anomaly. In the Iron Mine and Kaiserstuhl regions, suitable nonmagnetic factors that could be used as alternative cues appear to have been largely unavailable. Hence, it is not surprising that depriving the pigeons of magnetic input by using a strong magnet did not improve their orientation [18]. Even if previous experience with the Vogelberg anomaly did not help our birds at other sites within the anomaly (see Supplemental Information Part 4), the findings in the Iron Mine and Kaiserstuhl anomalies [13, 14, 18] can be reconciled with ours ([15] and present study).

Gagliardo and colleagues (e.g., [19, 20]), on the other hand, deprived pigeons of magnetic "map" information by severing the trigeminal nerve and found no effect on initial orientation and homing performance. These findings are in agreement with ours: at the control sites, we, too, rarely found a difference between untreated controls and experimentals. Our conclusions, however, differ. Gagliardo and colleagues concluded that trigeminally mediated magnetoreception is not involved in the navigational process in homing pigeons [19], yet their data only show that pigeons can cope with the loss of magnetic information at most sites. The behavior of our untreated control birds within the anomaly implies that magnetic factors are detected and regularly consulted, but their contribution to the navigational process seems to vary between sites. The differences in initial orientation and vanishing intervals between groups observed at site C4, together with the large pulse effect [16], suggest that magnetic "map" factors may play a more important role at this site than elsewhere.

The Function of the Receptors in the Beak

Our findings are the first to indicate the natural function of the receptors in the skin of the upper beak: they are indeed magnetoreceptors, with electrophysiological data [12] and theoretical considerations (see Supplemental Information Part 2) suggesting that they detect differences in magnetic intensity. The structure of these receptors is known in some detail from histological studies [3, 4]; considerations on the functions of these structures, however, had to rely largely on assumptions so far.

Subjecting passerine migrants and homing pigeons to a brief, strong magnetic pulse, a treatment that selectively affects magnetite-based receptors, causes these birds to deviate from the orientation of untreated control birds (e.g., [6, 16, 21, 22]), demonstrating that magnetite-based receptors are indeed involved in avian navigation, yet without directly indicating their specific role. Behavioral studies disrupting the information flow from the receptors in passerine migrants, pigeons, and domestic chickens have clearly shown that the receptors in the beak are not involved in the avian inclination compass [7, 23–25]. This is in agreement with their assumed role of providing "map" information. The positive evidence for their effect on behavior shows that they mediate the detection of a strong artificial field [5], the effect of the magnetic pulse mentioned above [6], and provide the directing information for the so-called "fixed direction" responses (see [7] for details) but does not reveal their natural role.

Our data now show an effect of these receptors in a natural environment. In the Vogelsberg anomaly, the magnetic conditions may be unusual, but it is a situation that birds can easily manage if they are used to it, as the normal density of the local bird populations indicates. Pigeons not used to the irregular field are initially confused when they encounter it; disrupting this information by anesthesia of the beak cancels this adverse effect. Our findings thus support the hypothesis that the receptors in the upper beak provide information on magnetic intensity [12] as part of the navigational "map." They appear to represent a "magnetometer," in contrast to the avian magnetic compass located in the eye [26, 27].

Experimental Procedures

The experimental releases were performed during the summer seasons of 2006 to 2009, from April to September, on sunny days with little or no cloud cover. All experiments were performed in accordance with the animal welfare laws and regulations of Germany.

Test Birds and Treatment

The test birds were adult pigeons at least two years of age. When young, they had participated in a standard training program up to 40 km in the cardinal compass directions; additionally, they had completed several training flights each spring. The vast majority had also homed singly a varying number of times from various directions and distances in previous experiments. Pigeons were released only at test sites from which they had never homed before.

The receptors in the upper beak were temporarily deactivated with the local anesthetic Xylocaine 2% (AstraZeneca, Wedel, Germany; active substance lidocaine hydrochloride), which was applied by soaking a cotton bud in the substance and gently rubbing it along the edges of the upper mandible. The anesthetic was applied twice: first before entering the magnetic anomaly, about 15 to 25 km before reaching the test site, to prevent the birds from sensing the irregular magnetic field, and again about 1 to 5 min before they were released. Experimental birds released at the control sites also had their beaks anesthetized at a similar distance from the release sites and again before release. The control birds were untreated.

Release Procedures

The release procedure was the traditional one: the birds were transported to the release site in a Volkswagen van, released singly (alternating experimental and control birds), and observed by two observers using 10 × 40 binoculars (Zeiss Dialyt) until they vanished from sight. The birds' vanishing bearings were recorded with the help of a compass to the nearest 5°, and the vanishing intervals were recorded with a stopwatch. We continued to release pigeons until we had obtained ten evaluable bearings per group per release.

Data Analysis and Statistics

To analyze initial orientation, we tested the mean vectors from both groups at each site via Hotelling's two-sample test [28]. To compare the behavior of experimentals and controls within and outside the anomaly, we used a two-way ANOVA with absolute deviation from the home direction, vector length, and median vanishing interval as dependent variables, and with treatment (anesthesia or not) and location (within or outside the anomaly) as independent variables. Bonferroni's multiple comparison test or, if the data were not normally distributed, Dunn's multiple comparison test was used as a post hoc test to look for significant differences between samples.

Supplemental Information

Supplemental Information includes Supplemental Results and Discussion, four tables, and one figure and can be found with this article online at doi:10.1016/j.cub.2010.06.073.

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