DOES THE AVIAN OPHTHALMIC NERVE CARRY MAGNETIC NAVIGATIONAL INFORMATION?

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Summary

The bobolink (*Dolichonyx oryzivorus*) uses the earth's magnetic field as one source of directional information for its migratory orientation. However, the location and structure of the magnetoreceptors that transduce the magnetic information to the nervous system are unknown. Because treatment with a strong magnetic pulse results in a change in the direction of orientation, one of the receptors is thought to involve a magnetizable material such as magnetite. The effects of the magnetizing treatment can be abolished (i.e. the bird returns to its original orientation) by blocking the ophthalmic branch of the trigeminal nerve, but the ability of the bird to select and maintain a direction is not affected. These results are consistent with the hypothesis that a magnetizable material such as magnetite is part of the magnetoreceptors that are associated with the ophthalmic nerve.

Key words: magnetoreception, magnetite, ophthalmic nerve, orientation, bobolink, *Dolichonyx oryzivorus*.

Introduction

The ability of animals to detect and utilize directional information from the ambient magnetic field has received increasing documentation. Much of the work in this field has been focused on the navigational abilities of homing and migratory birds (Wiltschko and Wiltschko, 1988; R. Wiltschko and Wiltschko, 1995). Of the various magnetoreceptor mechanisms that have been proposed for terrestrial animals, two (magnetite and photopigments) have received the greatest amount of attention (Beason and Semm, 1994). Experimental evidence supports the use of a wavelength-sensitive transducer (Wiltschko *et al.* 1993; W. Wiltschko and Wiltschko, 1995) and a magnetizable material (Wiltschko *et al.* 1994; W. Wiltschko and Wiltschko, 1995; Beason *et al.* 1995; R. C. Beason, R. Wiltschko and W. Wiltschko, in preparation) by birds for detecting the magnetic field. Two, apparently independent, magnetoreceptors have also been postulated for an amphibian, the eastern red-spotted newt (*Notophthalmus viridescens*), with at least one receptor being wavelength-sensitive (Phillips, 1986; Phillips and Borland, 1994).

It has been proposed that the two avian magnetoreceptor systems may serve different functions, with the wavelength-sensitive receptors serving the magnetic compass and the magnetite-based receptors serving the map system (Semm and Beason, 1990). Two species of migratory birds (the Australian silvereye *Zosterops lateralis* and the European robin *Erithacus*

rubecula) are disoriented when they are provided with only red illumination, but orient correctly under the other wavelengths tested (Wiltschko *et al.* 1993; W. Wiltschko and Wiltschko, 1995). These responses are consistent with the explanation that the avian compass is wavelength-dependent and presumably involves photopigments. The results from experiments on magnetizing migratory birds (Wiltschko *et al.* 1994; W. Wiltschko and Wiltschko, 1995; Beason *et al.* 1995) are less clear and could be interpreted to mean that either magnetic compass or magnetic 'map' receptors (or both) are based on a magnetic material such as magnetite. However, the data from magnetized homing pigeons clearly indicate that, although the treatment affected the birds' vanishing directions, it was not a magnetic compass that was affected (Wiltschko and Beason, 1991; R. C. Beason, R. Wiltschko and W. Wiltschko, in preparation). Neither can we rule out the possibility that these mechanisms are two components of a single complex system (W. Wiltschko and Wiltschko, 1995). It is thought that the wavelength-sensitive receptors are the retinal photoreceptors and the magnetite receptors are associated with the ophthalmic branch of the trigeminal nerve (Beason and Semm, 1991), but there is no direct evidence to support either conclusion.

The bobolink *Dolichonyx oryzivorus*, a New World transequatorial migratory bird, has been shown to use the geomagnetic field for directional information during its

migratory orientation (Beason, 1987, 1989, 1992). Although visual cues (star patterns in a planetarium) play a role in the orientation of this species, the magnetic field provides the primary compass information when there is a conflict with other sources of information (Beason, 1987, 1989). The magnetic compass functions in the same fashion as reported for other species, i.e. it is an inclination compass that distinguishes between 'equatorward' and 'poleward' directions rather than north and south (Wiltschko and Wiltschko, 1972). The objectives of these experiments were to test the hypotheses that the magnetite-based magnetoreceptor is associated with the ophthalmic nerve and that the information carried by this nerve is used for orientation. As in previous studies to investigate the transducer substance, we used a behavioural assay to determine the effects of the manipulation on the physiological system.

Materials and methods

For these experiments, a total of 14 bobolinks *Dolichonyx oryzivorus* (L.) were tested during the autumn migratory season in funnel-test cages (Emlen and Emlen, 1966). The birds were captured as adults using mist-nets. All birds were housed indoors without a view of the natural sky throughout the experiment. When the bobolinks exhibited migratory readiness, they were tested between 19:30 h and 21:30 h under the natural magnetic field within a planetarium (see Beason, 1989, for details). The cages were covered with translucent tops that precluded the birds from obtaining any visual cues outside the cage. The experimental protocol was the same as that described previously (Beason *et al.* 1995), but involved three conditions: control, magnetized and nerve-blocked. Because the objective of the experiment was to determine the effect of the treatments on the orientation of individual birds, each bird served as its own control. Each bird's modal nightly direction of activity was measured to the nearest $10\,^{\circ}$. The modal direction (the direction with the greatest amount of activity) was used as a measure of central tendency because it is easier to evaluate and does not differ significantly from the mean direction (Cherry and Able, 1986). During the control period, the directional preference of each bird was determined by testing it on four successive nights and calculating the mean of the nightly modal directions. All nightly responses to the experimental manipulations were calculated as deviations from that bird's control mean direction to reduce the effects of inter-individual variation in preferred headings (Beason, 1989). The effects of the treatments were assessed in an analysis of the second-order means. The significance of orientation for each pooled data set was tested with the Rayleigh test and means were compared with the control direction using confidence intervals (Zar, 1984). The means of treatments were compared using Watson's U^2-test (Zar, 1984).

Following the control period, each bird was magnetized north-anterior and tested on the following three nights. The birds were magnetized such that if the bill were iron, the tip would attract the south end of a compass (see Beason *et al.*

1995, for details of the magnetizing apparatus). In the final set of experiments, the ophthalmic nerve of the magnetized birds was blocked by the nightly application of a drop of Lidocaine (diluted 1:10 in saline) directly onto the nerve immediately before testing for three consecutive nights. Access to the nerve was gained surgically through a small incision anterior to the orbit made 2 days prior to the control portion of the experiment (three birds) or by insertion of a hypodermic needle through the skin covering the anterior part of the orbit (11 birds). For the surgical procedure, the birds were anaesthetized with a mixture of ketamine hydrochloride (Ketaset, $0.05\,\mathrm{mg\,g^{-1}}$) and xylazine (Rompun, $0.01\,\mathrm{mg\,g^{-1}}$). The nerve was blocked each night immediately before the birds were placed into the test cages at 19:30 h. The effectiveness of the nerve blocking was tested by tugging on the feathers of the forehead. If the bird flinched, additional anaesthesia was applied. Tugging the feathers was used as an indicator of successful anaesthesia because the ophthalmic nerve also receives sensory information from the mechanoreceptors associated with the feathers of the forehead. The birds were removed from the test cages at 21:30 h, before the effects of the anaesthesia dissipated. Because there were no differences in headings for any of the experiments between the birds whose ophthalmic nerve was exposed surgically and those in which the injection was made through the skin, the data for the two groups were pooled.

Results

The mean direction of the birds during the control portion of the experiment was seasonally appropriate (Fig. 1), towards the southeast ($\alpha=138\,^{\circ}$, $\mathbf{r}=0.854$, $P<0.001$, where α is the mean direction and $\mathbf{r}$ is the mean vector length). After being magnetized north-anterior, the birds exhibited a mean deviation of $77\,^{\circ}$ counterclockwise ($\alpha=283\,^{\circ}$, $\mathbf{r}=0.629$, $N=11$, $P<0.01$), which was significantly different ($P<0.01$) from the control direction (Fig. 2). When the ophthalmic branch of the trigeminal nerve of the magnetized bird was blocked by an injection of Lidocaine immediately prior to testing each evening, the mean deviation ($\alpha=13\,^{\circ}$, $\mathbf{r}=0.746$, $N=10$, $P<0.01$) of the birds was not significantly different ($P>0.05$) from the control direction, but differed significantly from their heading when the nerve was not blocked ($U^2=0.2945$, $P<0.01$). The reason for the differences in sample sizes in Fig. 2A,B is that some birds gave a generalized response to the Lidocaine application and became inactive, apparently sleeping, during the last experiment.

Discussion

It has been shown previously that the primary orientation cue used by bobolinks during the migratory season is the geomagnetic field (Beason, 1987, 1989, 1992). The mean direction selected by the birds in this experiment is the same as in previous experiments in which orientation cues were manipulated (Beason, 1989, 1992) and is seasonally

appropriate. The deviation produced by the magnetizing pulse is not significantly different (U^2=0.077, P>0.05) from that reported previously for birds magnetized in the same manner (Beason *et al.* 1995) and indicates that a magnetizable material (probably magnetite) is employed by the birds for magnetoreception during migratory orientation. This receptor appears to be associated with the ophthalmic nerve because the effects of the magnetization could be abolished by blocking the nerve. Consequently, the electrophysiological responses of ophthalmic nerve cells to magnetic stimulation (Beason and Semm, 1987; Semm and Beason, 1990) are biologically relevant and probably reflect the sensory capability of magnetic receptors associated with the magnetite deposits.

Wiltschko *et al.* (1994) reported that the effect of magnetization had diminished by the second day following treatment in silvereyes and was absent by the fourth day. In previous experiments with bobolinks, the effect of the magnetization persisted through the 4–5 days of post-treatment testing (Beason *et al.* 1995). Because the present experiments only lasted 6 days after treatment, the change in heading on day 4 is almost certainly caused by the nerve block and not by an accommodation to the magnetization.

These results could indicate that more than one type of receptor is used to obtain magnetic compass information, with the bird giving priority to the magnetite-based receptor of the ophthalmic nerve. When information from the ophthalmic

Fig. 1. Orientation of bobolinks during the autumn migration period. The mean direction for all birds was 138 ° (**r**=0.854, *P*<0.001, where α is the mean direction and **r** is the mean vector length). Mean vectors of individual bobolinks during the control portion of the experiment are drawn proportional to the radius of the circle and relative to magnetic north (at the top of the circle). Each dot on the periphery represents the respective mean heading of each bird, *N*=14.

nerve is unavailable (because of the blocked nerve in this case), the bird would have to rely exclusively on alternative compass receptors. If the sole magnetic compass receptor were affected by the nerve blocking, then the birds would not have been able to select and maintain a constant direction in the absence of visual cues. Because the Lidocaine-treated birds were not disoriented and showed the same heading as they did before being magnetized, they must have had a functioning compass that was not based on a magnetizable material. The only compasses that have been reported in migratory birds are visual and magnetic (Beason and Semm, 1991). When the visual cues (star patterns in a planetarium) and the natural or artificial magnetic field were oriented in opposite directions, bobolinks used the magnetic compass as their primary source of directional information (Beason, 1987, 1989). In the present experiments, it is unlikely that visual cues were being used for orientation because the cages were covered with translucent plastic tops that diffused the light and prevented the birds from discerning any objects outside their cages. The dim illumination (1.01x) was produced by bulbs that ringed the inside of the planetarium dome and reflected off the dome, resulting in uniform illumination. The test cages were distributed in a circle about the centre of the dome and positioned so that they were level with the widest point of the dome to prevent light/dark patterns or gradients in light intensity.

If the birds were not using visual cues for orientation when the ophthalmic nerve was blocked, then one explanation is that they were using another magnetic receptor. The retinal photoreceptors could potentially fulfill that role. The use of retinal photoreceptors for magnetic field detection would be consistent with the results of other experiments on vertebrates in which the direction selected for orientation was modified by the wavelength of the illumination (Phillips and Borland, 1992, 1994; Wiltschko *et al.* 1993; W. Wiltschko and Wiltschko, 1995).

Alternatively, if the bobolink uses a 'map and compass' in the same way as humans (Kramer, 1953), these results could be interpreted to mean that magnetite-based receptors associated with the ophthalmic nerve provided location ('map') information and another receptor using a nonferromagnetic transducer provided compass information. The sensitivity of the ophthalmic nerve cells meets the requirements of a receptor used for a magnetic map (Semm and
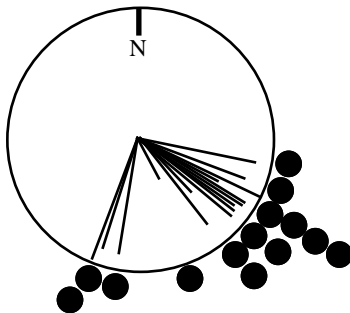
Fig. 2. Responses of individual bobolinks to treatment with a magnetic pulse and with Lidocaine relative to their individual control directions (indicated by the tick at the top of the circle). (A) Orientation of birds magnetized north-anterior (pooled mean: α=283 °, **r**=0.629, *N*=11, *P*<0.01). (B) Orientation of the same magnetized birds when their ophthalmic nerve was blocked with Lidocaine immediately prior to testing (pooled mean: α=13 °, **r**=0.746, *N*=10, *P*<0.01). The mean vector for each bird is drawn proportional to the radius of the circle. Each dot on the periphery represents the mean heading of one bird.
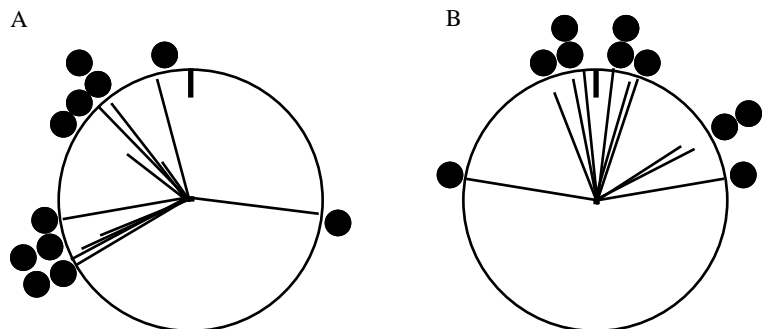
Beason, 1990). If the bird did not know where it was (its map was not functioning because the input was blocked by the Lidocaine), one strategy would be to adopt a heading in a seasonally appropriate direction (i.e. southeast in the autumn). Because all the birds in our experiment were captured as adults, they had made at least one migratory round trip previously and could have been 'goal-oriented' towards their non-breeding grounds. The responses of the magnetized bobolinks are what would be predicted for birds compensating for longitudinal displacement to the west (Beason *et al.* 1995). However, in order to compensate, they would require a functioning 'map'. The magnetic field has the theoretical potential to provide bicoordinate map information (comparing horizontal field intensity with vertical or total field intensity in the northeastern United States results in a grid intersecting at 20°), but whether it actually fulfills that function is unresolved (Gould, 1982).

The real proof of these conclusions can only come from navigation experiments in which the ophthalmic nerve is blocked and if map effects then disappear. In order to establish that it is a map factor which is affected, the bird's location and its goal must be known. The most logical experiment is to magnetize homing pigeons (Wiltschko and Beason, 1991; R. C. Beason, R. Wiltschko and W. Wiltschko, in preparation) and to test the effect of blocking the ophthalmic nerve on their homing abilities.

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