

Having the nerve to home: trigeminal magnetoreceptor *versus* olfactory mediation of homing in pigeons

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Summary

The ability of pigeons to find their way home from unfamiliar sites located up to hundreds of kilometers away is well known, but the mechanisms underlying this ability remain controversial. One proposed mechanism is based on the suggestion that pigeons are equipped with magnetoreceptors that can enable the detection of either the earth's magnetic field and/or magnetic field anomalies in the local terrain over which the pigeons fly. Recent reports have suggested that these magnetoreceptors are located in the upper beak where they are innervated by the ophthalmic branch of the trigeminal nerve. Moreover,

this nerve has been shown to mediate pigeons' ability to discriminate the presence *versus* the absence of a magnetic field anomaly in a conditioning situation. In the present study, however, we show that an intact ophthalmic branch of the trigeminal nerve is neither necessary nor sufficient for good homing performance from unfamiliar locations, but that an intact olfactory nerve is necessary.

Key words: navigation, magnetic sense, olfactory guidance, homing pigeon.

Introduction

Despite strong evidence that olfactory cues or an intact olfactory system are necessary for pigeons to find their way home from unfamiliar sites (Papi, 1991; Wallraff, 2004; Wallraff, 2005), an olfactory explanation of pigeon homing has failed to win universal approval, partly because it evokes 'intuitive incredibility' (Wallraff, 2005) and partly because of a belief in the primacy of other mechanisms, e.g. one based on magnetoreception (Wiltschko, 1996; Walker, 1998) such as is used in some other vertebrates (Walker et al., 1997). Moreover, it has been argued that some findings in favour of the role of olfaction in pigeon navigation could not be replicated (Wiltschko, 1996; Gould, 2004). Actually, a careful inspection of those results and the related experiments reveals that those inconsistencies concern more the initial orientation than the homing abilities of the anosmic pigeons and that the experimental protocols adopted were not standardised and did not guarantee the achievement of a total and long lasting anosmia of the treated birds. An exhaustive discussion on the importance of an appropriated methodology in testing a navigational hypothesis in homing pigeons has been published (Papi, 1986; Wallraff, 2005).

Recently, Mora et al. succeeded in conditioning pigeons to discriminate the presence *versus* the absence of a magnetic field anomaly, and showed that the magnetic perception was mediated by the ophthalmic branch of the trigeminal nerve

(V1) (Mora et al., 2004). The magnetic conditioned response was lost following bilateral resection of V1, but it persisted following bilateral resection of the olfactory nerve (ON). The involvement of the trigeminal nerve in magnetoreception was proposed following the observation of superparamagnetic particles in connection with the endings of the ophthalmic branch of this nerve (Fleissner et al., 2003; Williams and Wild, 2001). Due to the close proximity, in the upper beak, of V1 to ON, all previous studies that have reported a navigational impairment in anosmic pigeons (for references, see Wallraff, 2005) have been questioned, by presuming that any method used to make the birds anosmic, e.g. anaesthesia, application of zinc sulphate to the olfactory mucosa, nostril plugging and, in particular, olfactory nerve section, might have accidentally damaged or in some way affected the trigeminal endings involved in the magnetic perception (Mora et al., 2004).

As a consequence of this issue, it is necessary to directly compare the roles of the olfactory nerve and of the ophthalmic branch of trigeminal nerve in homing. Therefore, from two sites located in opposite directions with respect to home, we released three different groups of inexperienced homing pigeons subjected to: (1) a sham operation (SS), (2) bilateral section of the olfactory nerve (ON), or (3) bilateral section of the ophthalmic branch of the trigeminal nerve (V1).

Materials and methods

Subjects and releases

Seventy-two adult homing pigeons *Columba livia* L., born in the year of the experiment and housed in a loft at the Arnino field station [geomagnetic inclination 59.8°, total intensity (field strength) 46532 nT] 10 km SW from Pisa, Italy, were used. The birds were allowed spontaneous daily flights from their loft. At about 5–6 months of age, they were divided into three groups: bilateral section of ON ($N=24$), bilateral section of V1 ($N=24$), or sham surgery (SS, $N=24$; 12 sham ON, 12 sham V1). Half of each group of pigeons was released 4 days after surgery and the other half 8 days after surgery. The release sites, unfamiliar to the birds, were located in almost opposite directions with respect to home, i.e. Bolgheri in the south (geomagnetic inclination 59.4°, total intensity 46404 nT) and Marinella (geomagnetic inclination 60.2°, total intensity 46643 nT) in the north (see Table 1 for other details). In the experimental region, the isobars of the main geomagnetic parameters are all oriented along an east–west axis while the release sites were located along a north–south axis. Therefore, the releases were carried out with the greatest possible difference in the magnitude of the geomagnetic parameters between each release site and home. Both releases took place in sunny conditions, with no or very light winds. The birds were released singly, one from each group in turn, and their flight was observed using 10×40 binoculars. The azimuth of the vanishing bearing was recorded with a compass and vanishing and homing times were also recorded.

Surgery

The surgical procedures [approved by the Ethical Committee for Experimentation on Animals of the University of Pisa (C.A.S.A.)] were almost identical to those used in

Mora et al.'s study (Mora et al., 2004) and were performed by the same person (J. M. Wild). In the present study, each pigeon was anaesthetised with an intramuscular injection of 20% chloral hydrate (2 ml kg⁻¹ body mass) and fixed in a stereotaxic device with ear and beak bars. The olfactory nerve was sectioned bilaterally midway between the olfactory bulb and the point at which the nerves begin to diverge to pass to the olfactory epithelium, that is, rostral to the point at which the V1 crosses over the olfactory nerve. The right V1 was sectioned on the medial aspect of the eye following incision of the orbital fascia at the orbital rim and gentle depression of the globe. The left V1 was sectioned from the right orbit after a small hole was made in the semi-transparent bony inter-orbital septum, through which the nerve was pulled and cut. A 1–3 mm piece of nerve was removed in each case, and a drop of cyanoacrylate was applied to the cut ends to prevent re-apposition. Sham operations consisted of the full surgical approach, but the nerves were not cut.

Statistics

For each distribution of vanishing bearings a mean vector and homeward component were calculated; the latter ranges from -1 to +1 and gives an indication of the strength of the group's homeward orientation. The distributions of vanishing bearings were tested for uniformity by both the Rayleigh and the V-test, the latter taking into account the expected direction (Batschelet, 1981). The three experimental groups were compared by applying a non parametric analysis of variance (ANOVA; Kruskal–Wallis test). One between-group comparison was made on the absolute angular difference between the vanishing bearing of each subject and the mean direction of its group in order to test for group differences in dispersion. Another comparison was made on the signed

Table 1. Summary of homing results from pigeons released at two sites

Release site	Group	<i>N</i>	<i>n</i>	α	<i>r</i>	hc	vt (s)	hp (km h ⁻¹)
Bolgheri	SS	12	12	098°	0.18	-0.10	241"	2.7
	V1	12	10	245°	0.56*	-0.01	225"	6.6
	ON	12	11	183°	0.91***	-0.81	217"	Lost
Marinella	SS	12	12	173°	0.84***	+0.79***	169"	Day after
	V1	12	11	182°	0.85***	+0.75***	176"	6.6
	ON	12	11	145°	0.02	+0.02	273"	Lost
Pooled results	SS	24	24	031°	0.41*	+0.35**	218"	Day after
	V1	24	21	351°	0.39*	+0.39**	181"	6.6
	ON	24	22	208°	0.44*	-0.39	252"	Lost

Bolgheri release site: home direction 336°, distance, 54.8 km, date of release, 5/8/05; Marinella release site: home direction 154°, distance, 57.4 km, date of release, 13/8/05.

Groups: SS, sham operated control pigeons; V1, pigeons subjected to the resection of the ophthalmic branch of the trigeminal nerve; ON, pigeons subjected to the resection of the olfactory nerve.

N, number of birds released; *n*, number of birds for which the initial orientation was recorded; α , mean vector direction; *r*, mean vector length; hc, $r\cos(\alpha-\beta)$, where β is the home direction; vt, median vanishing time (s); hp, homing performance: median homing speed in km h⁻¹ is reported when available; 'day after' and 'lost' means that more than half the birds homed the day after the release, homed in the subsequent days or were lost; asterisks in the *r* and hc columns indicate the results of the Rayleigh and V-test respectively; * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

angular difference between the vanishing bearing of each subject and the home direction, to test for group differences in orientation with respect to the home direction (Wallraff, 1979).

Vanishing time and homing performance were compared by the Kruskal–Wallis ANOVA. Dunn's test was used for the *post hoc* analysis (Zar, 1984).

As each pigeon was released only once, we could pool the data of the two releases by setting the home direction to 360°. The analysis of the combined results was achieved using same statistical procedure as for the single experiments.

Results

The vanishing times of the three groups of birds were similar in the release from Bolgheri (Kruskal–Wallis, $P>0.5$), but significantly different in the release from Marinella (Kruskal–Wallis, $P<0.01$), the ON pigeons being slower than both the other groups (Dunn's test ON vs V1, $P<0.02$; ON vs SS, $P<0.05$). This difference was not revealed by a test on the pooled data (Kruskal–Wallis, $P>0.1$).

The distribution of vanishing bearings of the SS birds was significantly oriented in the release from Marinella ($P<0.001$ for both the Rayleigh and the V-test, which takes into account the expected direction), but not from Bolgheri ($P>0.5$ for both Rayleigh and V-tests). The V1 birds displayed significantly oriented distributions from both release sites according to the Rayleigh test (Bolgheri, $P<0.05$; Marinella, $P<0.001$) and in the release from Marinella according to the V-test (Bolgheri, $P>0.05$; Marinella, $P<0.001$). The ON birds, however, were significantly oriented at Bolgheri, but in the opposite direction to home (Rayleigh test, $P<0.001$; V-test $P>0.5$), and randomly scattered at Marinella ($P>0.5$ for both Rayleigh and V-tests).

The peculiar initial orientation of the three experimental groups from Bolgheri merits detailed discussion. In previous experiments at this site (Benvenuti et al., 1996; Ioalè et al., 2000; Gagliardo et al., 2001), pigeons have displayed a strong tendency to fly towards the preferred compass direction (PCD), which for Arnino pigeons is south–southwest (Ioalè, 1995; Ioalè, 1996). This tendency it is usually counterbalanced by the tendency to fly towards home for the control birds, while it prevails in the birds showing an impaired homing ability. This often produces an initial orientation towards west for the pigeons able to home, similar to what happened for the V1 pigeons, and an orientation towards south for the pigeons impaired in homing, similar to what happened for the ON pigeons. Therefore, while the orientation observed in both V1 and ON birds is consistent with previous data recorded at Bolgheri, the scattering of the SS pigeons is quite unusual.

A between-group difference in the orientation of vanishing bearing was observed from Bolgheri, but not from Marinella (Kruskal–Wallis, $P<0.01$ and $P>0.5$, respectively). In particular, from Bolgheri the ON birds were differently oriented from the SS pigeons (Dunn's test, ON vs SS, $P<0.005$; $P>0.05$ for the other comparisons). A significant between-

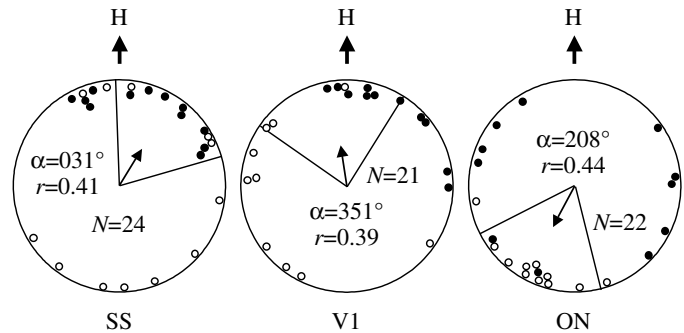


Fig. 1. Pooled distributions representing the initial orientation of the three experimental groups: SS, sham operated control pigeons; V1, pigeons subjected to the resection of the ophthalmic branch of the trigeminal nerve; ON, pigeons subjected to the resection of the olfactory nerve. The pooled distributions (mean vector direction, α , and mean vector length, r) were obtained by setting the home direction (H) to 360°. Each symbol represents the vanishing bearing of a single bird. The open and the filled dots represent the birds released from Bolgheri and Marinella, respectively. The outer arrow indicates the home direction, the inner arrow represents the distribution's mean vector. The inner lines delimitate the 95% confidence interval of the distribution.

group difference in dispersion was evident only in the release from Marinella (Kruskal–Wallis, Marinella, $P<0.0005$; Bolgheri, $P>0.1$), where the ON birds were significantly more scattered than both the other two groups (Dunn's test, ON vs V1, $P<0.001$; ON vs SS, $P<0.005$).

The analysis of the pooled data showed that both SS and V1 birds were significantly oriented according to both the Rayleigh and the V-test and that the 95% confidence limits of both mean vectors included the home direction (see Table 1 and Fig. 1). By contrast, the ON birds displayed a mean vector significantly oriented (Rayleigh test, $P<0.05$) towards the direction opposite to home (V-test, $P>0.2$); in fact, the 95% confidence limits of the mean vector did not include the home direction (see Table 1 and Fig. 1). Tests on the pooled data showed an overall between-group difference in vanishing bearing orientation (Kruskal–Wallis, $P<0.05$), the ON birds being significantly differently oriented from the SS pigeons (Dunn's test, $P<0.02$).

Irrespective of whether the data were analysed separately for individual releases, or as pooled data from combined independent samples, the results of the homing performances were similarly dramatic (see Fig. 2 and Table 1 for the median values of the homing times). At both release sites, the ON birds were significantly poorer at homing than both the V1 and SS pigeons (from Bolgheri: $P<0.001$ for both comparisons; from Marinella: ON vs V1, $P<0.001$; ON vs SS, $P<0.05$). Together the pooled results showed that only 4/24 ON birds returned home, compared with 23/24 V1 and 23/24 SS birds (Dunn's test $P<0.001$ for both comparisons). No statistical difference emerged from the comparison between the V1 and SS pigeons, although the V1 birds tended to home slightly faster than the SS birds.

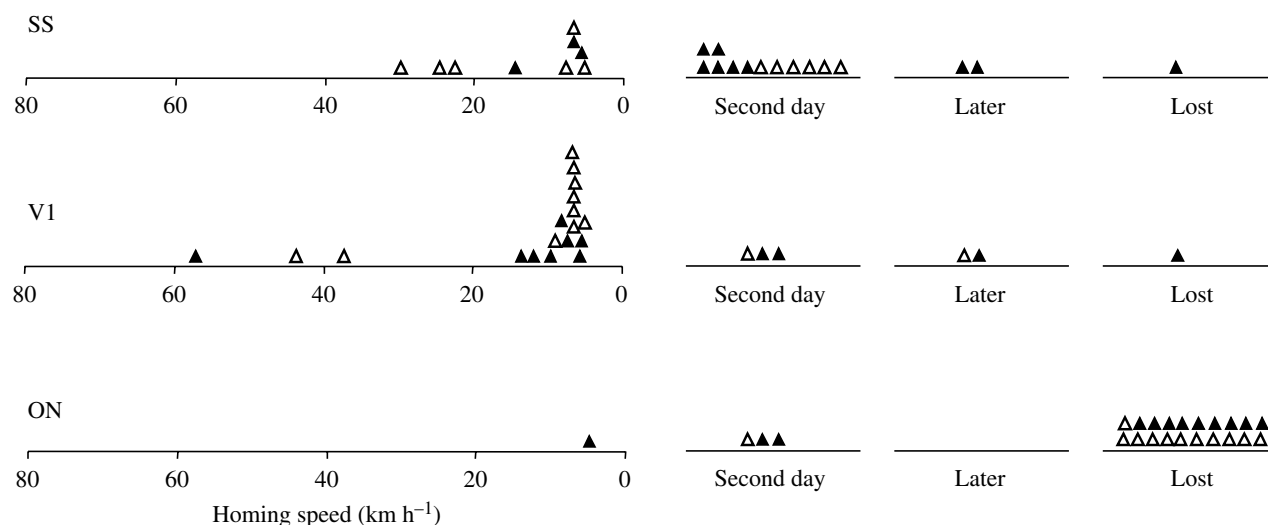


Fig. 2. Pooled homing performance of the three experimental groups (see Fig. 1). For the birds that homed on the same day of the test release, homing speeds are reported. Birds that homed on the second day, later and lost are also indicated. Open and the filled triangles represent birds released from Bolgheri and Marinella, respectively.

Discussion

Our experiment showed a dramatic impairment in the homing performance of pigeons with the olfactory nerve sectioned. In contrast, neither the sham operation nor resection of V1 affected the capability of pigeons to return to their home loft. These results of V1 *versus* ON section are exactly the reverse of those found by Mora et al. in a magnetic conditioning situation (Mora et al., 2004), a seemingly surprising result, especially since the surgeon and the surgical procedures were the same in both studies. However, although Mora et al.'s study (Mora et al., 2004) demonstrated the important finding that magnetoreception could be mediated by V1, it could not assess the role of magnetic detection in a situation in which position needs to be determined with respect to home. Furthermore, the completely negative effects of ON section in that study could, in retrospect, simply be seen as not surprising, since olfactory cues were presumably unimportant in the learning and performance of the magnetic conditioning task. In contrast, the present results show that a trigeminally mediated magnetic sense, thought to be transduced using magnetite in the upper beak (Fleissner et al., 2003; Mora et al., 2004; Williams and Wild, 2001; Winkelhofer et al., 2001), is not necessary for untrained pigeons to find their way home from unfamiliar sites.

A particularly important implication of the results of the present study is that the results of all the previous studies showing a navigational impairment in birds made anosmic by different methods, and whether trained or untrained, and tested from unfamiliar locations at various distances from home, cannot be attributable to possible damage of the trigeminal magnetoreceptor system (Mora et al., 2004), because in the present study the ON section was made proximal to the point at which V1 crosses ON and hence did not damage V1.

Moreover, since the ON birds did not appear to be aided in any way by an intact V1, the results also suggest that an intact V1 is not sufficient for homing under the present conditions, confirming the similar conclusions of others (for references, see Wallraff, 2005). However, the results of ON section do not, by themselves, show that anosmia is the root cause of the poor homing performance, because deafferentation of the olfactory bulb might have effects on homing performance unrelated to olfaction *per se*. This possibility was assessed and rejected by Papi et al. (Papi et al., 1980), who showed that homing was severely affected by unilateral olfactory nerve section combined with plugging of one of the nostrils, only if the cut and plug were on opposite sides, thereby producing olfactory impairment. Furthermore, a possible reduction in motivation to home is an unlikely explanation of the ON birds' poor homing performance in the present study because, in other studies (Wallraff, 1980; Wallraff et al., 1989), inexperienced anosmic pigeons have been recovered from long distances from either home or the release site, indicating their willingness to continue flying, despite being disoriented with respect to home. Moreover, the fact that anosmic pigeons released from familiar locations are as proficient as controls in orienting and homing argues against a motivational effect of anosmia (Wallraff et al., 1993).

These results not only strongly confirm several previous demonstrations of the profound effects of olfactory nerve section on pigeon orientation and homing (Papi, 1991; Wallraff, 2005), but they also provide no support for a specific magnetic map hypothesis based on magnetoreception mediated by trigeminal ophthalmic nerve fibres and terminals that are thought to be necessary for homing over unfamiliar areas (Williams and Wild, 2001; Winkelhofer et al., 2001; Fleissner et al., 2003). Our results do not, however, invalidate the possibility of magnetic detection mediated by V1 in laboratory

tests (Mora et al., 2004) and do not countermand the possibility that a magnetic sense can be used to determine compass directions (Wallraff, 2005) or to detect magnetic anomalies (Walcott, 2005), although pigeons do not seem to be consistently affected by them (Wiltschko and Wiltschko, 2003).

Although the present data are incontrovertible with respect to the effects of each of the nerve sections, it has been opined that atmospheric odours play a more significant role in pigeon homing in Italy than in other parts of the world (for references, see Wallraff, 2005), despite the fact that anosmic birds have been shown to be impaired in all the countries where the tests have been conducted (Europe, Africa, North and South America) (Benvenuti et al., 1998; Wallraff, 2005). But even if true, such geographical or environmental differences should not be regarded as some form of confounding variable; rather, the task is to determine which particular cues, or combination of cues, are operative in each particular location, as well as to determine the mechanisms that mediate perception of those cues. Some authors have proposed that when environmental conditions provide insufficient olfactory cues, pigeons develop the ability to rely on magnetic cues for navigation (Wiltschko et al., 1987; Walcott, 2005), but this still remains to be fully demonstrated. In fact, Benvenuti et al. (Benvenuti et al., 1990) were not able to confirm the results reported by Wiltschko et al. (Wiltschko et al., 1987) and a large body of evidence has shown that the lack of exposure of young pigeons to the winds carrying olfactory information impairs the development of navigational abilities (Wallraff, 1966; Gagliardo et al., 2001; Odetti et al., 2003).

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