

## Activational effects of odours on avian navigation

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The sensory basis of the navigational map remains one of the most important and intriguing questions in animal behaviour. In birds, odours have been hypothesized to provide the primary source of map information. Convincing tests have shown that experienced homing pigeons rely on map information obtained at sites where they are exposed to natural odours, even if subsequently released (without additional olfactory information) at a different site. These findings have been interpreted as support for the olfactory map hypothesis. Using this ‘false-release-site’ (FRS) approach, we compared the effects of exposure to natural odours with that of exposure to a series of artificial odours lacking spatial information. Our findings show that olfactory exposure to either natural or artificial odours at an FRS caused pigeons to rely on map information obtained at the FRS, even if subsequently released at the true-release site in the opposite direction from the home loft. Because artificial odours did not provide map information, however, the findings clearly demonstrate that olfactory exposure provides no navigational information to pigeons whatsoever; instead it activates an independent non-olfactory map system. This test decisively contradicts the olfactory map hypothesis, which predicts that olfactory cues are the primary source of navigational information used by birds.

**Keywords:** navigational map; olfactory activation; olfactory navigation; homing pigeons

### 1. INTRODUCTION

The map sense underlying long-distance homing ability remains one of the most important unresolved questions in animal behaviour. The olfactory map hypothesis proposes that the spatial distribution of natural odour sources provides the primary source of navigational information used by vertebrates as diverse as sea turtles and homing pigeons (Papi 1986, 2001; Wallraff 2004, 2005). However, recent experiments with young, inexperienced pigeons that rely on route-based cues to determine geographical position have shown that odours do not provide navigational information, but instead activate non-olfactory path integration systems (Jorge *et al.* 2009). These findings call into question an important prediction of the olfactory map hypothesis (i.e. that olfactory cues are used to keep track of the displacement route), but do not rule out the possibility that olfactory cues obtained at release sites provides navigational map information used by older, experienced birds.

Findings from dozens of experiments have been interpreted as support for the use of an olfactory map by experienced homing pigeons (Wallraff 2004, 2005). The most compelling of these experiments involve manipulation of natural wind-borne odours. In one such experiment, clockwise (CW) or counterclockwise (CCW) rotation of wind direction at the home loft by means of large deflector panels was shown to cause a corresponding CW or CCW shift in the initial orientation

of pigeons released at unfamiliar sites (Baldaccini *et al.* 1975), as predicted by the olfactory map hypothesis (Papi 1986). Contrary to the olfactory map hypothesis, however, other experiments have shown that the deflector loft effect is not due to wind-borne odours (Kiepenheuer 1978, 1979, 1982), but rather to light cues altered by the deflector panels (Phillips & Waldvogel 1982, 1988; Waldvogel & Phillips 1991). The light cues altered by the deflector panels are proposed to form part of a light-based reference system derived from polarized light patterns used to calibrate the avian sun compass (Phillips & Waldvogel 1988; Muheim *et al.* 2006; Phillips *et al.* 2006); olfactory input appears to be necessary to activate the acquisition and/or recall of this stored information.

A second type of experiment in which exposure to natural odours was manipulated, ‘false-release-site’ (FRS) experiments (Benvenuti & Wallraff 1985; Wallraff 2004, 2005), also provides a cornerstone of the olfactory map hypothesis. In these experiments, experienced homing pigeons without access to odours were transported to a ‘FRS’ where they were exposed to natural odours (figure 1*a*). The birds were then deprived of odours and transported to a ‘true-release site’ (TRS) in roughly the opposite direction from the home loft (figure 1*a*). Controls were transported directly from the home loft to the TRS and exposed there to natural odours. After anaesthetization of the olfactory mucosa (to prevent the acquisition of any additional olfactory information), birds from the two groups were released alternately at the TRS (Benvenuti & Wallraff 1985). Pigeons exposed to natural odours at the TRS were homeward oriented, while birds exposed to natural odours at the FRS oriented in the opposite direction,

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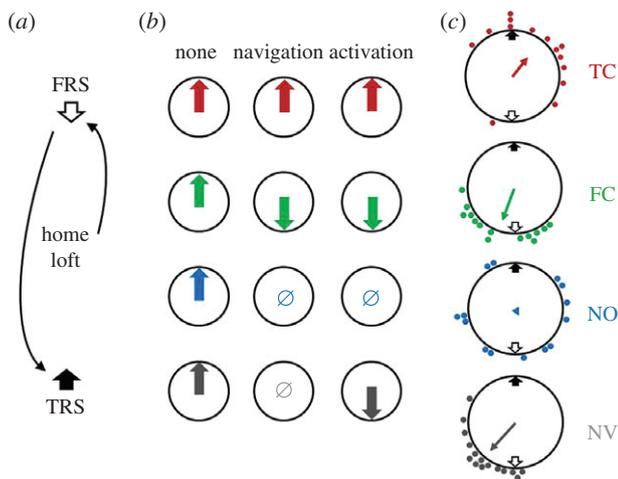


Figure 1. FRS experiment. (a) Schematic drawing of the outward journey from the loft to the TRS by way of the FRS. (b) Predicted directions of orientation according to none, navigation or activation effects of odours. (c) Colour arrows represent the predicted directions where mean vectors of each group should be pointed to.  $\emptyset$  indicates disorientation (i.e. failure to exhibit consistent directional orientation). TC, red; FC, green; NO, blue; NV, grey. (c) Black upward arrows, the true home direction; white downward arrows, the false home direction. Symbols plotted at the periphery of the circle show the vanishing directions of individual birds. For group colours, see (b). The arrows originating from the centre of the circle represent the mean vectors.

i.e. the homeward direction at the FRS. Similar findings were obtained when the FRS and TRS were reversed, indicating that the difference in orientation of the two groups was not due to the properties of a particular release site. These findings clearly show that pigeons obtain map information at the sites where they have access to natural odours, and are consistent with the predictions of the olfactory map hypothesis (i.e. access to natural odours enables pigeons to locate their position on an olfactory map), but are also consistent with an activation effect of olfactory cues (i.e. access to natural odours activates the use of non-olfactory map cues).

In the present experiments, we carried out a modified version of the FRS experiments (Benvenuti & Wallraff 1985). As in the original experiments carried out by Benvenuti and Wallraff, we used a true control (TC) group exposed to natural odours at the TRS and a false control (FC) group exposed to natural odours at the FRS; unlike the original experiment, our TC group was transported to the FRS along with the FC group, but the birds were only exposed to natural odours when they arrived at the TRS (figure 1a). In addition, we incorporated two other treatment groups. We tested a group of pigeons that received no olfactory information prior to release, no-odour (NO) birds, to control for non-specific effects of olfactory deprivation (figure 1b). Finally, we tested a group of pigeons that was exposed to novel odours (NVs) at the FRS to distinguish between activation or navigational effects of odours (figure 1b); NV birds were not exposed to natural odours at any time. Because we reversed the position of the TRS and the FRS between the two experiments (§2), while the same sequence of artificial/NVs was used, we ruled out the possibility of the NVs providing map information.

## 2. MATERIAL AND METHODS

FRS experiments were carried out with homing pigeons housed at our loft at the Herdade da Ribeira Abaixo field station (38°06'N; 8°36'W), 8 km South of Grândola, Portugal. Adult pigeons with previous release experience (2–5 years old) were placed in the experimental loft, and were forced to fly at least 1 h d<sup>-1</sup>. Also during this period, four flock releases were carried out from sites more or less in the cardinal directions at distances up to 5 km from the loft. The experimental loft (2 m × 2 m × 2 m) was at the ground level with four windows, one in each wall, that allowed a full view of the surrounding area. A commercial multi-grain food mixture was provided twice a day after the training flights, while water and grit was given ad libitum.

Two release sites were chosen in opposite directions at distances around 50 km from the loft. The two sites were chosen along the North–South axis because the proximity of the coast line to the west of the loft made it impossible to carry out symmetrical releases at these distances along the East–West axis. Experienced adult pigeons were divided into four groups and transported to the release sites inside airtight Plexiglas boxes (0.5 m × 0.5 m × 0.3 m). A TC group was transported in filtered air to the FRS, kept there in this condition for 1 h and then transported to the TRS in the same condition (filtered air). After arrival at the TRS, TC birds were allowed to smell natural air for 45 min before the start of the release. An FC group was transported in filtered air to the FRS and allowed to smell natural odours at that site for 45 min. Then, the birds were placed back into filtered air for 15 min and transported in this condition to the TRS (table S1, electronic supplementary material). At the TRS, FC birds were kept in filtered air until released (table S1, electronic supplementary material). An NV group was exposed to exactly the same sequence of treatments as the FC, except that at the FRS, instead of natural air, FC birds were exposed to artificial/NVs (a new odour was presented every 5 min; table S1, electronic supplementary material). Finally, an NO group was kept continuously in filtered air, i.e. during transport, at the FRS and at the TRS (table S1, electronic supplementary material). Ten to 15 minutes prior to release, birds from all four treatment groups had their nostrils anaesthetized with xylocain spray (AstraZeneca) to prevent them from obtaining any additional olfactory information when released (Benvenuti & Wallraff 1985; Wiltschko *et al.* 1987).

The experiment was carried out twice, using the same two sites, but the site used as the FRS in the first experiment was used as the TRS in the second experiment, and vice versa. In both replicates of the experiment, the NV group was exposed to the same sequence of artificial odours (lavender, lavender + camellia, camellia, camellia + eucalyptus, eucalyptus, eucalyptus + rose, rose, rose + jasmine and jasmine) at the FRS (Jorge *et al.* 2009). Pigeons used in the second set of releases were the returnees from the first set. However, in the second set of releases, pigeons from each treatment group in the first set were divided among the other three treatment groups.

To eliminate natural odours from the supplied air, outside air was filtered by means of a filter device equipped with two filters in series, a high-efficiency filter designed for clean room applications (class H14 extracts 99.995% of dust particles; dimensions 305 mm × 305 mm × 78 mm from TROX technik, model F782) that served as a pre-filter, and an activated carbon filter (TROX technik, model

Table 1. Homing performance. Group designations are defined in figure 1.  $n$ , number of valid bearings;  $\alpha_m$ , mean vector direction;  $r_m$ , mean vector length; asterisks indicate the significance by the Rayleigh test;  $\Delta h_T$ , deviation from the true home direction;  $\Delta h_F$ , deviation of the false home direction; asterisks indicate the significance by  $V$  test. Differences in the mean vanishing bearings between groups are presented and significances given by the Watson  $U^2$  test.

place	group	$n$	vector		$\Delta h_T$	$\Delta h_F$	vanishing time (min:s)	homed: first day, later, lost	homing velocity ( $\text{m s}^{-1}$ )
			$\alpha_m$	$r_m$					
<i>single releases</i>									
TRS	TC	8	54°	0.62*	77°	-118°	2:40	1, 5, 2	0.486
337°, 42 km	FC	8	193°	0.79**	-144°	21°*	2:18	2, 2, 4	0.887
FRS	NV	8	204°	0.78**	-133°	32°*	2:23	2, 3, 3	0.486
172°, 56 km	NO	8	212°	0.43	-127°	50°	2:36	1, 4, 3	0.486
TRS	TC	6	171°	0.83**	-1°*	-166°	2:57	1, 3, 2	0.648
172°, 56 km	FC	6	350°	0.79*	178°	13°*	3:04	1, 0, 5	5.185
FRS	NV	7	28°	0.90***	144°	51°*	3:14	2, 3, 2	0.648
337°, 42 km	NO	6	263°	0.49	91°	-74°	2:17	2, 0, 4	5.770
<i>pooled releases</i>									
true home	TC	14	38°	0.55*	38°*	—	2:47	2, 8, 4	0.567
direction 0°	FC	14	200°	0.75***	-160°	—	2:26	3, 2, 9	5.076
	NV	15	222°	0.83***	-138°	—	2:32	4, 6, 5	0.648
	NO	14	152°	0.17	152°	—	2:24	3, 4, 7	0.486
false home	TC	14	218°	0.65**	—	-142°	—	—	—
direction 0°	FC	14	17°	0.79***	—	17°*	—	—	—
	NV	15	42°	0.83***	—	42°*	—	—	—
	NO	14	350°	0.25	—	-10°	—	—	—
<i>comparison between all group mean vanishing bearings</i>									
	TC-FC	TC-NV	TC-NO	FC-NV	FC-NO	NV-NO			
release 1	139**	150*	158*	11	19	8			
release 2	179*	-143**	92	38	-87	-125**			
pooled releases	162***	-176***	114*	22	-48*	-70**			

\* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

F760; dimensions, external diameter 145 mm, length 250 mm) that removed the remaining odours. The filter box was made of medium-density fibreboard (inner measures 306 mm  $\times$  306 mm  $\times$  800 mm). Outside air was pumped into the filter box using the outflow of an MDA vacuum cleaner (model VC 4006) that was powered by an Einhell generator (model STE, 850). Air was supplied at a minimum rate of 500 l h<sup>-1</sup> per box (Wallraff *et al.* 1980; Jorge *et al.* 2009). At the release sites, an extension cord was used to bring power to the apparatus approximately 30 m away from the car and the power generator. The airtight boxes in which the birds were transported and held at the FRS and TRS were connected to the filter using a common vinyl garden hose, which kept the boxes 10 m away from the vacuum cleaner.

Releases were carried out under sunny conditions. Birds were released singly, one from each group in turn, and their flight observed using 10  $\times$  40 binoculars until they vanished from sight. The vanishing bearings were recorded to the nearest 5°, and the vanishing times to the near second. Homing times were recorded by a second observer at the home loft.

Directional data were analysed for each sample and were characterized by the mean vector (direction  $\alpha_m$  and length  $r_m$ ), the deviation from the home direction ( $\Delta h$ ) and the homeward component ( $h$ , where  $h = r \times \cos \Delta h$ ). The Watson  $U^2$  test was used to compare the angular dispersion of vanishing bearings in the four treatment groups in each release. The differences in the median vanishing

intervals and the homing times were evaluated using the Mann-Whitney  $U$  test (Batschlet 1981; Zar 1999).

### 3. RESULTS AND DISCUSSION

The initial orientation and homing performances of the four treatment groups in the two releases are shown in table 1, and the pooled data in figure 1c and table 1. In the two releases, the TC birds exhibited consistent orientation relative to the home direction, while NO birds failed to orient. Both FC and NV birds oriented in roughly the opposite direction from that of TC birds. The mean vanishing bearing of the TC birds differed significantly from the FC birds and from the NV birds, both in the individual releases and in the combined data from the two releases (table 1), while there were no significant differences between the FC and the NV birds either in the individual releases or in the data from the two releases combined (table 1). The NO birds differed from the TC birds in one experiment and from NV birds in another. The combined data showed differences among all groups, with the exception of the FC and NV birds (table 1).

As in the earlier FRS experiments, an analysis of the returning performance (i.e. homed: 1st day; later; lost; table 1) did not show significant differences (contingency table 3  $\times$  4,  $\chi^2_{(1)} = 0.04$ ,  $p > 0.05$ ). Nor were there differences in the vanishing times (Mann-Whitney  $U$  test,  $p > 0.05$  for all comparisons) or in the homing

speed (Mann–Whitney  $U$  test,  $p > 0.05$  for all comparisons).

The failure of NO birds to orient confirms the importance of olfactory information in navigation of homing pigeons, while the response of the TC and FC birds replicates the FRS effect originally demonstrated by Benvenuti & Wallraff (1985), in which the vanishing bearings of pigeons exposed to natural odours at the TRSs were homeward oriented, while those of pigeons exposed to natural odours at the FRSs (when subsequently released at the TRS) were oriented away from home (figure 1c). These findings confirm that experienced adult pigeons obtain map information at the site(s) where they are exposed to natural odours. However, they do not distinguish between the olfactory map (Papi 1986, 2001; Wallraff 2004, 2005) and olfactory activation (Jorge *et al.* 2009) hypotheses (figure 1b).

Although the olfactory map hypothesis and the olfactory activation hypothesis make similar predictions for the TC, FC and NO treatments (figure 1b), the predictions of these two hypotheses clearly differ for the NV treatment (figure 1b). If natural odours provide experienced pigeons with the primary source of site-based map information (Wallraff 2004), then NV birds (like NO birds) should be deprived of map information. If the olfactory map hypothesis is correct, the vanishing bearings of NV birds should fail to exhibit a consistent orientation relative to the home direction, and should be indistinguishable from those of the NO birds (figure 1b). In contrast, if the olfactory activation hypothesis is correct, exposure to NVs should have the same effect as exposure to natural odours. Therefore, the initial orientation of NVs birds (exposed to NV at the FRS) should be indistinguishable from that of FC birds (exposed to natural odours at the FRS), and the orientation of both these groups should be in a direction roughly opposite to that of the TC group (exposed to natural odours at the TRS). The findings in figure 1c clearly show that the orientation of the NV birds is consistent with the predictions of the activational hypothesis, and not the navigational hypothesis (figure 1b; see also Wallraff *et al.* 1986).

Consistent with the earlier experiments with young, inexperienced pigeons (Jorge *et al.* 2009), the present results indicate that olfactory input plays a critical role in homing by experienced adult pigeons, but does not provide a source of map information as proposed by the olfactory map hypothesis. Thus, olfactory activation of responses to non-olfactory cues explains the results of the three experiments that have been interpreted as providing the strongest support for the olfactory map hypothesis, i.e. olfactory deprivation experiments (Jorge *et al.* 2009), deflector loft experiments (Phillips *et al.* 2006) and FRS experiments (reported here). Furthermore, re-examination of these experiments failed to yield evidence that odours provide spatial/navigational information during any stage of the homing process, i.e. at the home loft (Phillips *et al.* 2006), during displacement (Jorge *et al.* 2009) or at release sites (present experiments), contrary to the key predictions of the olfactory map hypothesis. Remarkably, ruling out olfactory cues only deepens the mystery concerning the sensory basis of the map component of homing because the remaining alternatives (i.e. magnetic,

Wiltshcko & Wiltshcko 1996; Lohmann *et al.* 2004; Freake *et al.* 2006; and infrasound, Yodlowski *et al.* 1977; Hagstrum 2000 maps) explain some, but not all, cases of long-distance homing (Gould 1982, 2008; Lohmann *et al.* 2004; Freake *et al.* 2006).

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