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ORIGINAL ARTICLE

Olfactory information from the path is relevant to the homing process of adult pigeons

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Abstract
The contribution of path information to the homing process of adult experienced pigeons is by no means consensual. Adult pigeons are expected to determine the homeward direction solely based on information gathered at the release site (i.e., map information). However, several are the evidences suggesting that path information is somehow important to the homing process. Using adult homing pigeons and manipulation of the pigeons' access to olfactory information from the displacement to the release site by means of nasal plugs that were applied unilaterally, we accessed the contribution of olfactory path information to the homing process. Pigeons transported to the release site with only the right nostril plugged were impaired on the determination of the initial homeward direction, while pigeons transported to the release site with only the left nostril plugged were impaired on the homing speed. The present findings highlight the importance of information from the path to the navigational process wherein olfactory path information contributes to the accurate determination of the homeward direction and in keeping the pigeon's motivation to fly home.

Significance statement
Animal migration has fascinated mankind for generations. However, and although vast research has been done on the subject, it is still controversial the way as animals accomplish such endeavor task. Using homing pigeons, a biological model for spatial processing, and behavioral experiments for manipulation of information from the path (here, olfactory), we showed that information from the path contributes to the accurate determination of the initial homeward direction and to keep the pigeon's motivation to fly home. Therefore, information from the path (here, olfactory) appears to lie at the heart of the processing of spatial information in migrating animals, contributing to the accurate determination of flying course and motivation to reach the destination.

Keywords Path information · Homing pigeons · Unfamiliar sites · Odors · Motivation · Redundancy

Introduction

Path integration represents one of the easiest strategies for animals to keep track of their movements when traveling, or were passively displaced, through the environment (Griffin 1952; Bingman and Cheng 2005). Nevertheless, reliance on information of the path has generally been viewed as a transient stage in the development of the pigeon's navigation system, attributed to the inexperience of young birds and/or to the inaccuracy of the navigational map in the early phase of its development (Wiltschko and Wiltschko 1982, 2000; Tögel and Wiltschko 1992; Jorge and Vicente 2006; Jorge et al. 2006, 2008).

Findings from previous experiments in which homing pigeons were displaced by alternative routes to the same release site ("detour" experiments) provided compelling evidence that (1) pigeons take into account information from the path when making navigational decisions (Papi et al. 1973; Tögel and Wiltschko 1992; Jorge et al. 2006, 2008) and (2) for a transient stage in the development of the pigeon's navigational map (Tögel and Wiltschko 1992; Jorge and Vicente 2006; Jorge et al. 2006). The "detour" effect characterized by an attempt of

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pigeons to compensate for deviations occurred during the displacement to the release site is evident on the vanishing bearings of young inexperienced homing pigeons when the first part of the outward journey was intentionally deviated from the beeline between the release site and the home loft by ~90° (Tögel and Wiltschko 1992; Jorge and Vicente 2006; Jorge et al. 2006, 2008). Importantly, adults and/or experienced pigeons were not affected by deviations along the displacement to the release site, possibly because these pigeons discard information of the path when taking the initial decision about the homeward direction (Tögel and Wiltschko 1992; Jorge and Vicente 2006; Jorge et al. 2006).

Conversely, experiments in which researchers used unilateral nasal plugs during both the outward journey and return flight provided evidence that reliance on olfactory cues (i.e., odors from the path and return flight) was age-independent, i.e., both young and adult pigeons showed more scattered vanishing directions from the release site when the right nostril, but not the left nostril, was obstructed (Gagliardo et al. 2007, 2011). Interestingly, the pattern reversed when homing speed is considered, i.e., here left nostril obstructed birds (i.e., both young and adults) were slower than right nostril obstructed birds (Gagliardo et al. 2007, 2011). The findings were interpreted as evidence that pigeons rely on olfactory map information gathered at the release site or during the returning flight rather than to impairment on the acquisition of olfactory information from the path with relevance to the navigational process (Jorge et al. 2017). However, because the experimental treatment was not restricted to the release site and the return flight, being also applied during the outward journey to the release site, an effect of the absence of olfactory information from the path cannot be excluded.

The role of olfactory cues in pigeon navigation is controversial: (i) some have proposed that olfactory trace gases form gradients that can be used as maps (Papi 1986; Wallraff 2005), (ii) others that odors form a mosaic map in the vicinities of the home loft (Papi et al. 1973; Papi 1986), and (iii) others that odors activate neuronal circuits involved in homing (Jorge et al. 2014, 2017; Phillips and Jorge 2014). Nevertheless, redundancies in the avian navigation have been suggested as an advantage for animals to surpass less optimal conditions (Wiltschko and Wiltschko 1994; Walcott 2005; Beason and Wiltschko 2015). Therefore, the use of multiple cues or sources of information, including information from the path (e.g., landmarks or others geo-referenced information), by adult pigeons should not be completely excluded.

Here, homing pigeons were used as model organisms to investigate the role of olfactory cues from the path in the long-distance navigation of birds. We show that previous effects reported as evidence that pigeons rely on olfactory map information acquired only at the release site and the return flight (i.e., olfactory gradient map) are in fact due to the deprivation of relevant olfactory information from the path (i.e., landmarks or others geo-referenced information) used by birds to determine the homeward direction.

Methods

Twenty-two adult homing pigeons were tested on their homing abilities after have been transported to three distinct unfamiliar release sites (Vimieiro: distance, 81 km, direction, 290°; Comporta: distance, 75 km, direction 359°; and Alvaiazere: distance, 84 km, direction 200°), in one of the following conditions: control (C), pigeons were transported to the release sites with any nostril plugged; left plug (LP), pigeons were transported to the release site only with the left nostril plugged; right plug (RP), pigeons were transported to the release site with only the right nostril plugged (Fig. 1a). After arriving at the release site, all plugs were removed and birds had at least 30 min to experience natural odors from the local surroundings. Because we do not want to damage the pigeon's olfactory mucosa, plugs were made of cotton that fills up the nasal cavity. The plug was then held on its position with the help of adhesive tape.

Pigeons were released singly, alternated between groups, and followed with 10 × 40 binoculars until they vanished from sight. The vanishing bearing was recorded with a compass, and the vanishing time was recorded with a stopwatch. Homing times were also recorded by a second observer at the home loft.

Directional data were analyzed for each sample and were characterized by the mean vector.

The significance of the mean vector was determined via Rayleigh test. Differences in the median vanishing times and the homing times of the three groups were evaluated via Mann-Whitney U test. The number of pigeons lost was compared using the Fisher exact test.

For pooled data analysis, the significance of the homeward direction was given by the 95% confidence interval of the mean vector. Moreover, the significance of the mean vector length was given by means of bootstrapping technique. Here, 100,000 simulations were used to generate the 95, 99, and 99.9% confidence intervals of the significant vector length.

To minimize observer bias, blinded methods were use when all behavioral data were recorded and analyzed.

Data availability All data recorded are available as electronic supplementary material.

Results

In all releases performed at the three unfamiliar places, adult pigeons transported to the release site with the right nostril plugged showed impairment on the initial determination of

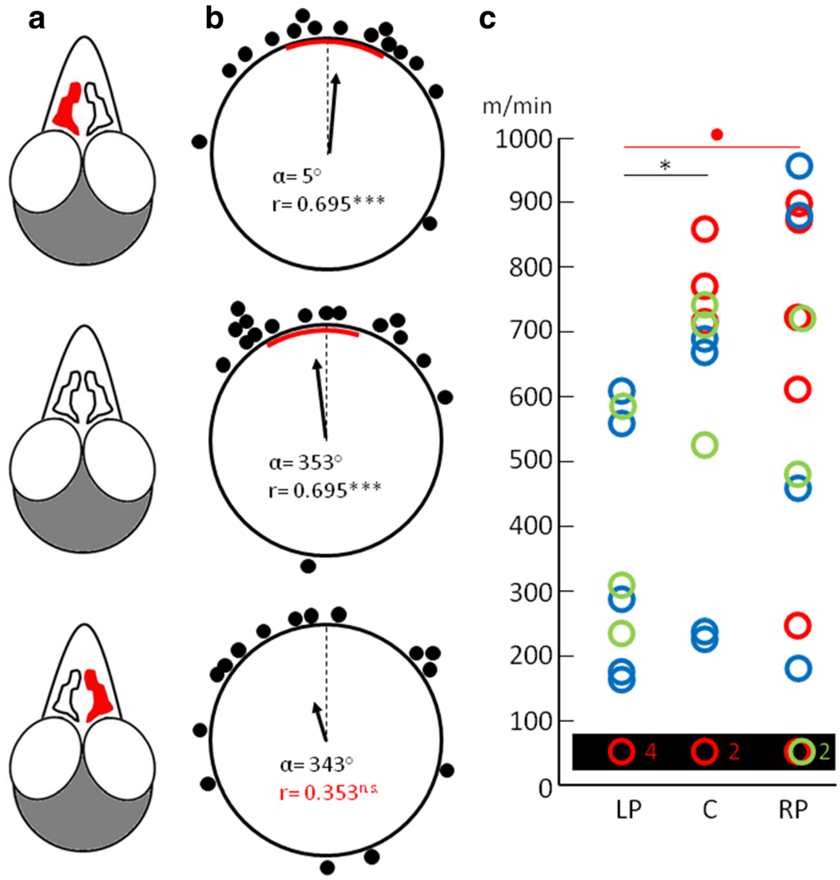

Fig. 1 Olfactory outward journey information on homing. **a** Schematic draw of nasal plugs (in red) applied during the transport of pigeons to the release site. RP, right plug; C, control with no plug; and LP, left plug. **b**, Circular diagrams are a pull representation of all individual vanish bearings having home as reference. Arrows irradiating from the center of the circumference represent the mean vector with a direction α and a concentration r; the red semi-circle represents the 95% confidence interval of the significantly concentrated mean vector. **c** Homing velocity expressed in meters per minute. Open circles, individual homing velocities of birds released at Vimieiro (Red), Comporta (Blue), and Alvaiazere (Green). Circles in the black box, pigeons returned on the 2nd day or later. Significance between median homing velocities is given by the Mann-Whitney test. $^{\bullet}p = 0.058$; $*p < 0.05$; $***p < 0.001$; ns not significant

the homeward direction compared to birds transported to the release site with no nasal plugs or birds transported with the left nostril plugged (in all three releases, the mean vector direction and the mean vector length of birds with the right nostril plugged were not significant; Table 1). This effect becomes more evident when the results of the three releases were combined (Fig. 1). Pigeons with the right nostril plugged were not significantly oriented (Fig. 1b), contrasting with birds with the left nostril plugged and birds with no plugs; in both cases, the 95% confidence interval of the mean vanishing direction included the direction of the home loft (Fig. 1b). In addition, vanishing directions of pigeons with the right nostril plugged were significantly more scattered than pigeons with the left nostril plugged (Fig. 2a) or pigeons with no plug (Fig. 2b). Moreover, although the birds with the right nostril plugged were significantly more confused, there was no difference

Table 1 Homing performances. *N*, number of pigeons released; *n*, number of valid bearings; α, mean homeward direction; *r*, concentration around the mean direction; *V.T.* median vanishing time given as (minutes:seconds), *H.T.* median homing time given as (hours:minutes), *Sig.*, significance given by the Mann-Whitney test. Significant concentration around the mean vanishing direction is given by the Rayleigh test. Asterisks give significance according to: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; *ns* not significant

Site	Group	*N*(*n*)	α	*r*	V.T.	Sig.	H.T.	Sig.	Lost
Vimieiro 290° 81 km	Control	6(6)	309	0,74*	3:46	ns	1:53	*	1
	R. Plug	6(5)	309	$0{,}46^{ns}$	3:30		2:02		–
	L. Plug	6(5)	313	0,91**	3:01		2nd day		2
Comporta 359° 75 km	Control	6(6)	332	0,93***	3:26	ns	3:32	ns	2
	R. Plug	6(6)	336	$0{,}45^{ns}$	3:48		2:03		2
	L. Plug	6(6)	336	0,82*	2:55		4:18		1
Alvaiazere 200° 84 km	Control	4(4)	186	$0{,}43^{ns}$	2:33	ns	1:57	ns	1
	R. Plug	4(4)	129	$0{,}32^{ns}$	3:30		13:27		–
	L. Plug	4(4)	233	$0{,}51^{ns}$	2:49		4:32		1

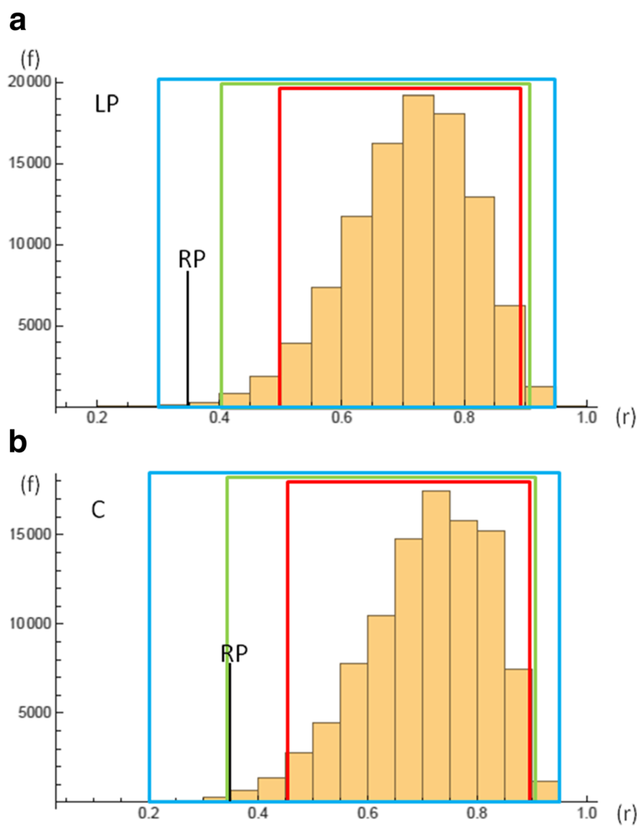

Fig. 2 Histogram of the outputs of the bootstrapping analysis (100,000 simulations). Confidence intervals (CI) for the mean vector length were calculated: Red box, 95% CI; green box, 99% CI; and blue box 99.9% CI. Y-axis, frequencies (f); X-axis, mean vector length (r). **a** Pigeons with the left nasal plugged (LP); and **b** pigeons with no plugs (C). The black bar labeled "RP" marks the length of the mean vector for birds with the right nasal plugged

respectively to the vanishing times of all released groups (Table 1).

Significant differences were found among the homing performances of the three groups (Fig. 1c). The pigeons with the left nasal plugged needed considerable extra time to arrive home than pigeons with the right nostril plugged or pigeons with no nasal plugs (Mann-Whitney test: $Z = -2.188$, $p = 0.058$ and $Z = -2.266$, $p < 0.05$, respectively). However, considering the extra time needed for birds with the left nostril plugged to arrive home, there was no significant difference in the number of birds lost during these experiments (Fisher exact test: $p > 0.05$ for all comparisons; Table 1).

Discussion

In the present study, applying nasal plugs unilaterally to adult homing pigeons, only during displacement to unfamiliar release sites (Fig. 1a), revealed that previously reported effects were primarily due to manipulation of relevant olfactory information during the outward journey, rather than at the release site or the return flight (Gagliardo et al. 2007, 2011). The initial orientations (i.e., vanishing bearings) of pigeons that only received olfactory input to the left nostril (right nostril plugged) during the displacement to the release site were randomly distributed (Fig. 1b and Table 1), indicating that they did not have acquired sufficient information during the outward journey and prior to release to accurately determine the home direction. In contrast, the faster homing speeds of this group (comparable to that of controls that received olfactory input to both nostrils; Fig. 1c) suggest little, if any, decrease in the motivation to home. Together, these two findings (i.e., fasting homing speeds despite the failure to orient in the homeward direction based on information gathered prior to and during the release; Fig. 1b, c) suggest that birds that only received olfactory input to the left nostril (right nostril plugged) during the outward journey were relying on information obtained at the release site and during the return flight (probably extracted from a gradient "map"; Fig. 1b, c).

In contrast, the vanishing bearings of pigeons that received olfactory input to both nostrils and those that received olfactory input to the right nostril (left nostril plugged) during the outward journey were accurately oriented in the homeward direction (Figs 1b and 2). However, birds that received only right nostril input (left nostril plugged) during the outward journey appeared less motivated to home, showing significantly lower homing speeds than either of the other two groups (Fig. 1c and Table 1). In an earlier study, GPS records showed that adult pigeons with the left nostril plugged stopped more often during the return flight than birds with the right nostril plugged or with neither nostril plugged (Gagliardo et al. 2011).

However, because in previous experiments with nasal plugs applied unilaterally, the experimental treatment was not restricted to the release site and return flight (i.e., having also been applied to the displacement), the findings gave rise to different interpretations, including (i) evidence for impairment on the acquisition of olfactory information from a gradient "map" (Gagliardo et al. 2007, 2011), (ii) evidence for impairment on the acquisition of olfactory information from the path (e.g., landmarks or others geo-referenced information), or (iii) evidence for impairment on the pigeon activation/motivation for homing (Wiltschko 1996; Jorge et al. 2014, 2017; Phillips and Jorge 2014). A motivation/activation effect has been previously proposed, as the primary effect for odors on pigeon navigation, activating neuronal circuitries not necessarily involved in olfactory processing (Jorge et al. 2014; Phillips and Jorge 2014).

More importantly, the findings reported here indicate that olfactory input to the right and left nostrils had different effects on homing behavior (Fig. 2), influencing the pigeon's reliance on information from the path and their motivation to maintain sustainable homeward flight (respectively). In fact, several neurophysiological studies have drawn attention to an asymmetrical processing of olfactory information with relevance to the navigation process (Gagliardo et al. 2005; Jorge et al. 2010, 2016, 2017; Patzke et al. 2010). Furthermore, the findings suggest that adult birds were able to determine the homeward direction using both (i) outward journey (path-based) information, i.e., birds with olfactory input to the right nostril (left nostril plugged) that exhibited homeward-directed initial orientation, and (ii) "map" information obtained after release, i.e., birds with the right nostril blocked that were initially disoriented but after leaving the vicinity of the release site were able to accurately determine the home direction as shown by homing speeds comparable to controls without nasal plugs (Fig. 1b, c). Redundancy in the use of spatial information has been previously shown in birds, particularly with respect to the use of compass information, where at least, the earth's magnetic field and the stars provide redundant information (Wiltschko and Wiltschko 1999; Muheim et al. 2006; Chernetsov 2015). Altogether, the present findings suggest that adult pigeons use redundant information to initially determine the homeward direction (i.e., path and map information), and that olfactory cues from the path are relevant for pigeons to keep their motivation for homing during the return flight.

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Author contributions PEJ designed the experiments. PEJ and BVP carried out the experiments, analyzed the data, and wrote the paper.

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Compliance with ethical standards

Ethical approval All the procedures were performed in compliance with the Portuguese and European animal welfare laws. This study was approved by the Portuguese Veterinarian Institute under the reference PTDC/BIA-BEC/99416/2008.

Competing interests The authors declare that they have no competing interests.

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