GPS tracking technology and re-visiting the relationship between the avian visual Wulst and homing pigeon navigation

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ABSTRACT

Within their familiar areas homing pigeons rely on familiar visual landscape features and landmarks for homing. However, the neural basis of visual landmark-based navigation has been so far investigated mainly in relation to the role of the hippocampal formation. The avian visual Wulst is the telencephalic projection field of the thalamofugal pathway that has been suggested to be involved in processing lateral visual inputs that originate from the far visual field. The Wulst is therefore a good candidate for a neural structure participating in the visual control of familiar visual landmark-based navigation. We repeatedly released and tracked Wulst-lesioned and control homing pigeons from three sites about 10–15 km from the loft. Wulst lesions did not impair the ability of the pigeons to orient homeward during the first release from each of the three sites nor to localise the loft within the home area. In addition, Wulst-lesioned pigeons displayed unimpaired route fidelity acquisition to a repeated homing path compared to the intact birds. However, compared to control birds, Wulst-lesioned pigeons displayed persistent oscillatory flight patterns across releases, diminished attention to linear (leading lines) landscape features, such as roads and wood edges, and less direct flight paths within the home area. Differences and similarities between the effects of Wulst and hippocampal lesions suggest that although the visual Wulst does not seem to play a direct role in the memory representation of a landscape-landmark map, it does seem to participate in influencing the perceptual construction of such a map.

1. Introduction

Homing pigeons (*Columba livia*) represent an important model species for understanding the neural basis of spatial behaviour, especially navigation while homing [1]. A large body of evidence has shown that homing pigeons possess an odour-based position finding mechanism (the so called olfactory map corresponding to the map step of Kramer's "map and compass" model [2]), allowing them to determine the direction of displacement relative to home on the basis of environmental odour cues at a release site [3–7]. Nevertheless, the olfactory map becomes redundant when pigeons are released within previously flown-over areas. In fact, while pigeons prevented from smelling local odours at the release site consistently display impaired navigational ability at unfamiliar locations, anosmic pigeons are unimpaired at navigating home from familiar locations because of their ability to exploit spatial information derived from familiar visual

landscape/landmark features [8–10]. Further, experimental data have shown that the olfactory map does not provide sufficient navigational information to efficiently guide homing in the vicinity of the loft [11, 12]. Therefore, pigeons rely on familiar landscape topographical information memorised during their previous, free flights within the home area for localising the loft during the final step of the homing process (local navigation around the loft) [11].

A vast body of experimental data have shown that the avian hippocampal formation (HF) is unimportant in supporting pigeons orienting homeward and approaching the home area when released from distant, unfamiliar locations, but is critically involved in familiar landscape/landmark-based navigation [13–18]. In fact, HF-lesioned pigeons are impaired navigating within the home area (4–6 km around the loft), where they have to rely on familiar topographical information to localise the loft [16,19,20]. However, given that landmark-based navigation is reliant on the visual recognition of familiar landmarks and

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topographical landscape features, it is surprising that identifying the crucial visual processing regions of the avian brain supporting navigation over familiar areas has been largely overlooked.

The avian telencephalon has two main targets of ascending visual processing streams from the thalamus, the visual Wulst and the entopallium, which are the projection fields of the thalamofugal and tectofugal visual pathways, respectively [21,22]. The Wulst is a laminated forebrain structure organized into two different functional regions, a smaller, anterior somatosensory region and a larger, posterior region processing visual information [23]. The visual Wulst is composed of four laminae: running from dorsal to ventral the hyperpallium apicale (HA), interstitial nucleus of the hyperpallium apicale (IHA), hyperpallium intercalatum (HI), and densocellular part of the hyperpallium (HD) [24, 25]. It is worth noting that HA and HD have connections with the HF [23,26,27]. Importantly, with respect to visually guided navigation, it is notable that the visual Wulst, and thalamofugal pathway in general, is thought to play a necessary role in processing information from the monocular lateral visual fields of each eye engaged in the viewing of distant (far field) objects [28–31]. There is additional evidence to suggest that the Wulst also supports visually guided cognition such as reversal learning and performance on spatial-operant tasks [32,33].

Surprisingly, few studies have specifically investigated the role of the thalamofugal pathway in birds challenged to use the lateral/far visual field for solving visual-spatial tasks in large, open field experimental settings. Experiments on pigeons trained in large arenas have shown an involvement of the Wulst in sun-compass mediated spatial learning and pattern discrimination tasks [28,34]. Watanabe and colleagues [35] trained zebra finches in an aviary to localise a rewarded feeder on the basis of either pattern (the four feeders were visually different) or spatial cues (the four feeders were visually indistinguishable but in constant locations). This experiment showed that an intact Wulst was required for solving the task using spatial cues.

In summary, the visual Wulst processes lateral, far field visual information, plays a role in visual cognition and has strong reciprocal connections with the HF. This promotes the hypothesis that the visual Wulst is critical in enabling pigeons to use visual landscape and familiar landmarks for navigation. Nonetheless, to date the only homing experiments assessing a possible role of the Wulst in navigation were performed on homing pigeons before the use of animal tracking technology. The relatively crude vanishing bearing and homing performance data recorded in these older experiments highlighted no or little effect of Wulst lesion on pigeon homing. The earliest experiments suggested that Wulst lesions in pigeons released from unfamiliar as well as familiar locations had no effect on homing performance [13,14,36]. However, the results of a latter study [37] suggested that the Wulst might have a role in familiar landmark-based navigation. Whether the avian Wulst may play a role in homing pigeon navigation remains largely unknown.

The current study provides the first in depth investigation into the possible role of the avian visual Wulst in homing pigeon navigation. By using state-of-the-art flight tracking technology and flight path data analyses [16–18,38], and for the reasons described above, we expected to observe changes in the flight behaviour of Wulst-lesioned pigeons that would be reflective of Wulst participation in the visual guidance of the homing pigeon landscape/landmark map, for example, diminished route fidelity or attention to linear landscape features.

2. Materials and methods

2.1. General procedure

Thirty-eight one year old homing pigeons (body weight 400–450 g), bred and housed at the Arnino Field Station (43°39′26″N, 10°18′14″E) of the Department of Biology, University of Pisa, Italy, were used in the experiment. Their previous homing experience consisted of two single releases of about 30 km from home to the east and south. The birds had access to food, water and grit ad libitum and were allowed to perform spontaneous flights around the home loft. The experimental procedures employed in the present study were approved by the Scientific Ethics Committee of the University of Pisa and authorised by the Italian Ministry of Health (permit number 185/2022-PR). To habituate the pigeons to carrying GPS data loggers (see below), a few weeks before the experimental releases the pigeons were equipped with a PVC dummy weight (45×25×15 mm, approximately 30 g) attached with a Velcro® strip (30×70 mm) glued to trimmed feathers of their back.

Before the experimental releases, 21 pigeons were subjected to bilateral ablation of the anterior forebrain visual Wulst (W group), and 17 birds were assigned to the control group, subjected to anaesthesia only (C group). After the surgery, the birds recovered for about ten days in their home loft with no post-surgical opportunity to fly out from the loft before being subjected up to 6 experimental releases from each of three release sites: La Costanza (43°48′22″ N, 10°20′43″ E; home direction 191°, home distance 17 km), Livorno (43°33′30″ N, 10°21′15″ E; 340°, 11.6 km), and Arnaccio (43°39′17″ N, 10°27′43″ E; 271°, 12.8 km). Each flight path was recorded using a GPS data logger, Mobile Action I-gotU (weight 22 g). The pigeons were transported to a release site following a standard procedure of being held in a crate, which was open to the air on top and also on one side from which they had a full view of the surroundings both during transportation and while at the release site. The pigeons were released singly (every 5–10 minutes) under sunny conditions with no or light wind. Each bird was subjected to one release per day. The within-series release site order varied across birds.

2.2. Surgery

The pigeons subjected to lesions of the Wulst were deeply anaesthetised with an intramuscular injection of chloral hydrate (20%, 2 ml/kg body weight) and then placed in a stereotaxic apparatus. Under a surgical microscope, a bilateral aspiration of the Wulst was performed. As the HF extends anteriorly up to about A8.0 (following the atlas of the pigeon brain by Karten and Hodos [39]), we were cautious to avoid causing any peripheral damage to the HF. Therefore, the targeted region extended from A9.0 to A13.0 up to a depth of 3 mm from the brain surface. The control birds were subjected to total anaesthesia as a sham procedure. The Wulst lesions were performed over a 2 day period.

2.3. Quantitative analyses

All the recorded tracks were visually inspected using QGIS (http://www.qgis.org). The GPS devices recorded positional data at 1 Hz. Sequential fixes reflecting flight speeds lower than 5 km/h were excluded from the analyses, as they corresponded to periods when the birds either stopped or were flying around without progressing in their journey. For the analyses we subdivided the tracks into three parts corresponding to distinct spatial/navigational phases of a homing flight as described in [9]: 1) decision making (DM), which reflects the decision-making process of a bird before a consistent navigational decision is taken while leaving the release site. It includes the portion of the track before a bird moves away from the release point, after having reached 2 km from the release site for the last time. The first fixes of the track, just after a pigeon is tossed into the air, up to when the bird first reached 0.5 km from the release point were excluded from this analysis; 2) the en-route phase (ER), which starts after the DM phase and lasts until a pigeon approaches for the first time the home area (4 km buffer radius around home); 3) the local navigation phase (LN), which starts when a pigeon enters for the first time within the 4 km radius from home and extends up to 500 m from the loft [9,11].

Although pigeons were released singly, some pigeons joined during the flight home. Tracks or sections of tracks from "joined" flights were excluded from the analyses. The flight was considered joined when pigeons flew at a maximal distance of 80 m within 10 seconds.

2.3.1. Homing performance and initial orientation during the first release from each site

In order to evaluate possible differences between the two experimental groups in homing success, the number of homed and lost pigeons from the first three releases (one from each site) was compared between the control and Wulst-lesioned groups by means of a χ^2 test. In addition, for each release site the homing performance of the control and Wulst-lesioned pigeons was compared by using the Mann-Whitney U test: we ranked the homing performance of the birds on the basis of the time recorded by the GPS in case this was still functioning; in case the GPS tracking data were not available, we assigned the two lowest ranks to the pigeons that homed later and to the lost pigeons, respectively.

For the pigeons that homed we computed the number of stops per km of track and the percentage of fixes associated to a flight speed lower than 5 km/h reflecting the time that the birds spent landed rather than flying. The Mann Whitney U test was used to compare these two parameters between treatments.

In order to assess the navigational performance of the control and Wulst-lesioned pigeons when released for the first time at each of the three test sites, we averaged the direction taken by a bird moving from one fix to the next during the DM phase only. For each release site the mean vector distributions of the control and Wulst-lesioned pigeons were tested for randomness with the One-Sample Hotelling test [40]. Between-group comparisons for each release site were performed with the Two-Sample Hotelling test [40].

2.3.2. Flight path efficiency

The Efficiency Index (EI) measures the general straightness of a path. It is computed as EI=b/l, where b is the beeline distance between the first and the last fix of a section of the track considered for each navigational phase, and l is the length of the flight path taken. The efficiency of the flight path was measured at different resolutions for each navigational phase. An Efficiency Index was computed both on the original recorded track (EI) and on a track obtained by computing a mobile mean of Latitudes and Longitudes across 40 consecutive fixes (EImm).

Specifically, the EI reflects both low spatio-temporal resolution flight path changes, corresponding to large deviations from the straight, home-loft directed flight path, and high spatio-temporal resolution flight path changes, representing the small but frequent deviations from a straight flight path. Being computed on a smoothed track, the EImm is designed to reveal the large detours from a straight flight path. In contrast, the ratio between the calculated EIs (R_{EI}=EI/EImm) gives a measure of the high spatio-temporal resolution flight path changes, as R_{EI} tends toward 1 if a bird displayed few small changes of direction [17].

Concerning the first three releases (one from each site), the analysis of the path efficiency during the three navigational phases was performed on the available tracks of the pigeons that homed at least once and was done to assess the effect of Wulst lesions on pigeon homing from unfamiliar locations. Concerning the analyses of the birds' performance across the repeated releases, the flight path efficiency was analysed on the routes belonging to pigeons released from each site at least three times and up to a maximum of six times.

For the first three releases (one from each site), possible differences between control and Wulst-lesioned pigeons in path efficiency (EI, EImm, R_{EI}) during the three navigational phases (DM, ER and LN) were assessed using a Generalised Linear Mixed Model (GLMM) with beta error distribution using treatment, experience level (from release 1–3) and interaction between treatment and experience level as fixed factors. Subject and release site were considered as random factors.

We tested the differences in flight efficiency between the Wulst-lesioned and control pigeons across repeated releases. We performed a GLMM with beta error distribution on the efficiency indexes (EI, EImm, R_{EI}) for each navigational phase (DM, ER and LN). For the DM and ER phases treatment, experience level (from 1 to 6 for each of the three sites), release site and interactions between treatment and experience level, and between treatment and release site were used as fixed factors.

Subject was considered as random factor. For the LN phase, we used treatment, experience level (from 1 to 18 corresponding to each release regardless of the site) and their interaction as fixed factors, while subject and release site were used as random factors. In the analysis of the EImm relative to the section of the tracks recorded in the ER and LN phases, the observation-level random effect was used for modelling the overdispersion [41]. The tracks with the LN portion interrupted and the tracks of joined birds, even if partially joined, were excluded from the path efficiency analysis.

2.3.3. Individual route fidelity

In order to assess the individual development of route fidelity, i.e. the tendency of a pigeon to fly a similar path to the loft during repeated releases, from the second to the last release from each site we computed for each bird the percentage of Fidelity Fixes (FF, fixes closer than 100 m from any fix of any previous track) for the en-route and local navigational phases together, as performed for analyzing the development of route fidelity in hippocampal ablated birds [18]. Using the same method for assessing the route fidelity allowed us to discuss possible differences/similarities between the effects of hippocampal lesions and lesions to the Wulst.

To measure possible differences in route fidelity development between control and Wulst-lesioned pigeons, we performed a GLMM to compare the percentage of FF for the en-route and local navigational phases combined, as performed in [18]. A GLMM with beta-binomial error distribution was used taking into account treatment, experience level (from 2 up to 6 for each site), release site, interactions between treatment and experience level, and between treatment and release site, as fixed factors. Subject was used as a random factor.

For the local navigation phase only, we assessed the overall tendency of a pigeon to fly over previously flown areas by computing a Spatial Occupancy Index as described in [17]. Using QGIS, for each fix of each individual's track, we determined a buffer of a 100 m radius around that fix. We then merged all the buffers of the fixes to obtain an area that was considered familiar and used that to assess flight fidelity on subsequent flights. For each individual, the Spatial Occupancy Index (SOI) was computed by dividing the merged/cumulative buffer area of all its tracks by the sum of the buffer areas of each single track. The SOI is included between 0 and 1 (0 > SOI > 1), being smaller in cases of maximal overlap of an individual's tracks. When the index tends to 1 the overlap is minimal, as the pigeon display more scattered flight paths.

The SOI scores computed for the track sections recorded during the LN phase were compared between the two groups by means of a Mann-Whitney U test.

2.3.4. Attraction to linear landmarks/landscape features

The attraction to linear landscape features was investigated independently for the three homing phases (DM, ER and LN). The leading lines were identified within the topographical features of the landscape over-flown by pigeons, by inspecting the tracks of each pigeon and creating a layer with the linear features followed at least once by at least one pigeon (Figure S1, Supplementary material). To assess whether control and Wulst-lesioned pigeons differed in their tendency to fly along the identified linear landscape features, we calculated the percentage of fixes closer than 100 m from any landscape leading line for each track (LL). Sequences of fewer than 20 fixes in a row (corresponding to less than 20 sec) were not considered in the analysis to exclude fixes associated with linear feature crossing.

To test whether the attraction to landscape leading lines is different between the two groups we ran a GLMM model for each phase to compare the percentage of LL. For the DM and ER phases, a GLMM with beta-binomial error distribution was performed for each phase using treatment, experience level (from 1 to 6; each level corresponds to one release from each of the three sites), release site, the interactions between treatment and experience level and between treatment and release site, as fixed factors, and subject as a random factor. For the LN

phase, the model was a GLMM with beta-binomial error distribution using treatment, experience level (from 1 to 18), their interaction and R_{EI} as fixed factors, subject and release site as random factors. In each model comparing the percentage of LL the observation-level random effect was used for modelling the overdispersion [41].

2.3.5. Statistical analyses

The linear mixed models were performed with R 4.1.2 [42].

The GLMMs were fitted with the R-package 'glmmTMB' ver 1.1.2.3 [43]. Fixed factors significance was tested using the Wald χ^2 test of the 'car' R-package ver. 3.0–12 [44]. Model assumptions were checked with the R-packages 'DHARMa' ver. 0.4.4 [45] and 'performances' ver. 0.10.2 [46]. We tested the occurrence of collinearity among predictors calculating the variance inflation factors (VIF) by means of the R-package 'performances' ver. 0.10.2 [46]. By means of the R-package 'emmeans' ver. 1.7.4–1 [47], the pairwise post hoc comparison was performed. To plot the effects of the models the R-package 'ggeffects' ver. 1.3.2 [48] was used.

2.4. Histology

Histology on the brains of the Wulst-lesioned pigeons was subsequently performed to assess the extent and the site of the lesion damage. The available Wulst-lesioned pigeons at the end of the homing experiments (N=5) were sacrificed with an overdose of chloral hydrate. The brains were extracted and fixed in a solution of 4% paraformaldehyde and then stored in 25% sucrose. The brains were cut coronally (section thickness: 20 μm) with a cryostat. The sections were stained with cresyl violet and the lesions reconstructed with the help of the pigeon brain atlas [39].

3. Results

The datasets generated and analyzed during the current study are available in the Movebank Data Repository (www.movebank.org), https://doi.org/10.5441/001/1.326 [49].

3.1. Homing performance and initial orientation during the first release from each site

The homing success of the two groups of birds during the first three releases (one from each site) was not significantly different (8 out of 21 W pigeons and 5 out of 17 C birds did not home; $\chi^2 = 0.31$, p > 0.5). However, while the homing performances of the two groups was comparable in the release from Livorno (see Table 1 for details; Mann Whitney test, U = 90, p > 0.1), which is the closest release site to the home loft, from both Arnaccio and La Costanza (U = 51, p < 0.01 and U = 76.5, p < 0.02, respectively) the Wulst-lesioned pigeons were significantly slower at homing than control birds.

The number of stops/km performed by control and Wulst-lesioned pigeons (computed for the birds of which the track was available, see n in Table 1) was never significantly different (Mann Whitney U test, AR U = 68.5, p > 0.2; CO U = 58, p > 0.05; LI U = 60.5, p > 0.05; see median number of stops/km in Table 1). However, the percentage of fixes associated to a speed lower than 5 km/h was significantly greater for the Wulst-lesioned pigeons compared to controls from the two further release sites (Mann Whitney U test, AR U = 37, p < 0.01; CO U = 34.5, p < 0.005) while from the nearest release site no difference emerged between the two treatments (LI U = 74.5, p > 0.2; see rt(%) in Table 1).

Both control and Wulst-lesioned pigeons during their first release from each of the three sites displayed individual mean vector distributions different from random in the DM phase from each release site (One Sample Hotelling test p < 0.01 in all cases, for details see Table 1 and Fig. 1). Controls and Wulst-lesioned pigeons displayed comparable mean vector distributions at both La Costanza and Livorno (Two Sample Hotelling test, p > 0.1). In fact, both groups were significantly oriented towards home from Livorno (their confidence intervals included the home direction, see Table 1 and Fig. 1), but oriented in a direction significantly different from the home direction from La Costanza. At Arnaccio, the Two sample Hotelling test highlighted a difference between the mean vector distributions of the control and Wulst-lesioned groups (p < 0.05). In fact, while control birds were more likely to fly southwest along the Arnaccio Road (Fig. 2), Wulst-lesioned pigeons exhibited more diverse directional preferences associated to less consistent individual orientation as suggested by their shorter individual mean vectors (see also Fig. 1). Therefore, while the Wulst-lesioned group was homeward oriented, the control birds displayed an orientation significantly different from the home direction. For the first release from Arnaccio, the LL (the parameter expressing the tendency to fly along leading lines; see paragraph 3.6 for further analyses) relative to Arnaccio Road was significantly greater for the control pigeons compared to the Wulst-lesioned pigeons (Mann-Whitney test, U = 12, p < 0.05; C n = 9, LL median (first and third quartiles), 0.93 (0.73–0.97), W n = 9, 0.32 (0.22–0.40)).

3.2. Flight path efficiency: first release from each site

Control and Wulst-lesioned pigeons displayed significant differences in their flight path efficiency during both DM (GLMM; C n = 15, EI estimated marginal mean ± se, 0.47 ± 0.19, W n = 21, 0.32 ± 0.18, p = 0.002; see Table S1, Supplementary material) and ER phase (EI, C n = 15, 0.66 ± 0.12, W n = 18, 0.54 ± 0.10, p = 0.001; see Table S1). The diminished path efficiency in Wulst-lesioned pigeons compared to control birds was due to both larger low-spatial resolution (DM-phase, EImm, C 0.69 ± 0.22, W 0.56 ± 0.20, p = 0.02; ER-phase, EImm, C 0.79 ± 0.15, W 0.71 ± 0.13, p = 0.01; see Table S1) and high-spatial resolution tortuosity (DM-phase, R_{EI}, C 0.66 ± 0.12, W 0.55 ± 0.11, p <

Table 1

Initial orientation. T, treatment; release site, name of the release site and home direction and distance from home; N (n), number of birds released (available tracks); α, mean vector direction; r, mean vector length; 95% CI, confidence intervals; L, number of lost birds; hp, homing performance expressed as median homing time; S, median number of stops/km; rt(%), median rest time expressed as the percentage of fixes associated to a speed lower than 5 km/h.

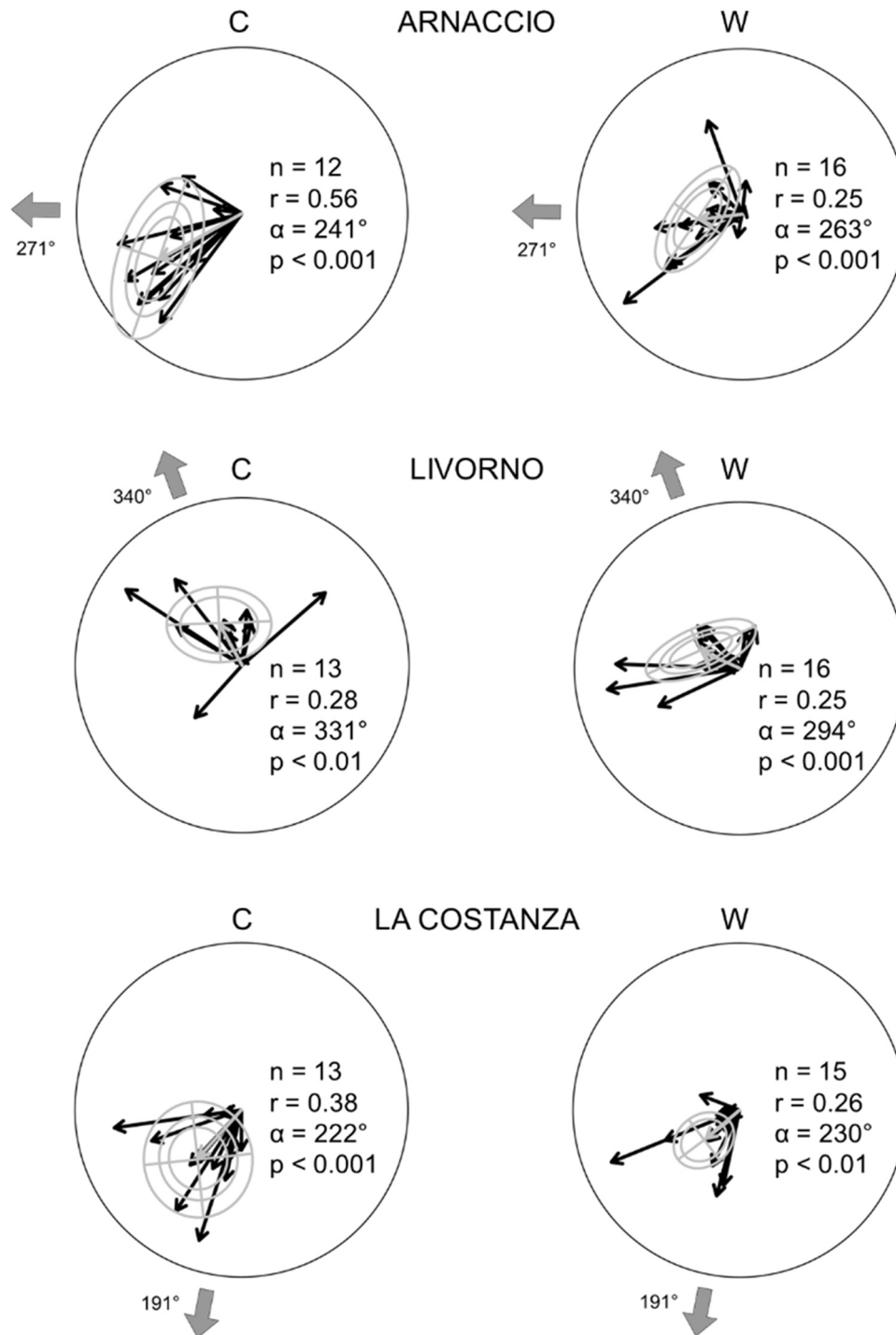| T	release site	N (n)	α	r	95% CI	L	hp	S	rt(%)
C	Arnaccio 271°, 12.8 km	15 (12)	241°	0.56	225°-268°	2	1 h 39 m	0.09	70
	La Costanza 191°, 17 km	15 (13)	222°	0.38	195°-250°	2	3 h 20 m	0.05	75
	Livorno 340°, 11.6 km	16 (13)	331°	0.28	292°-025°	1	1 h 23 m	0.04	73
W	Arnaccio 271°, 12.8 km	20 (16)	263°	0.25	236°-317°	1	6 h 47 m	0.13	89
	La Costanza 191°, 17 km	20 (15)	230°	0.26	201°-258°	5	16 h 36 m	0.14	91
	Livorno 340°, 11.6 km	20 (16)	294°	0.25	270°-344°	2	6 h 02 m	0.09	85

Fig. 1. Initial orientation. Mean vector distributions derived from the decision making portions of the tracks of the first release from each site. In each diagram, the outer arrow represents the home direction; the inner black arrows represent individual mean vectors; the inner grey arrow represents the second order mean vector of the distribution. The confidence ellipses correspond to the Hotelling test significance levels (p <0.05, p < 0.01, p < 0.001, from the smallest to the largest). The 95% confidence interval of the mean vector distributions (see Table 1 for details) can be visualised by drawing the tangents of the 95% confidence ellipse.

0.001; ER-Phase, R_{EI}, C 0.82 ± 0.09, W 0.76 ± 0.07, p = 0.003; see Table S1).

In contrast to what was observed following hippocampal lesions [16, 20] (see Discussion), Wulst lesions did not impair the ability of the birds to localise the home loft within the home area. In fact, neither EI (GLMM; LN-phase, C n = 15, EI estimated marginal mean ± se 0.71 ± 0.16, W n = 18, 0.65 ± 0.16, p = 0.13; see Table S1) nor EImm, which reflects low-spatial resolution tortuosity (C 0.82 ± 0.20, W 0.81 ± 0.19, p = 0.82; see Table S1), differed between control and Wulst-lesioned

pigeons during the first three releases. However, the Wulst-lesioned pigeons displayed greater high-spatial resolution tortuosity, as revealed by the comparison between R_{EI} of the two groups of pigeons (C 0.85 ± 0.11, W 0.81 ± 0.10, p = 0.02; see Table S1).

Considering the performance of the birds in the LN phase during the very first post-lesion release, regardless of the release site, we observed no differences between Wulst-lesioned and control pigeons in the efficiency indexes considered (Mann-Whitney test; EI, U = 59.5, p > 0.1, C n = 14, median (first and third quartiles) 0.78 (0.57–0.82), W n = 12, 0.63
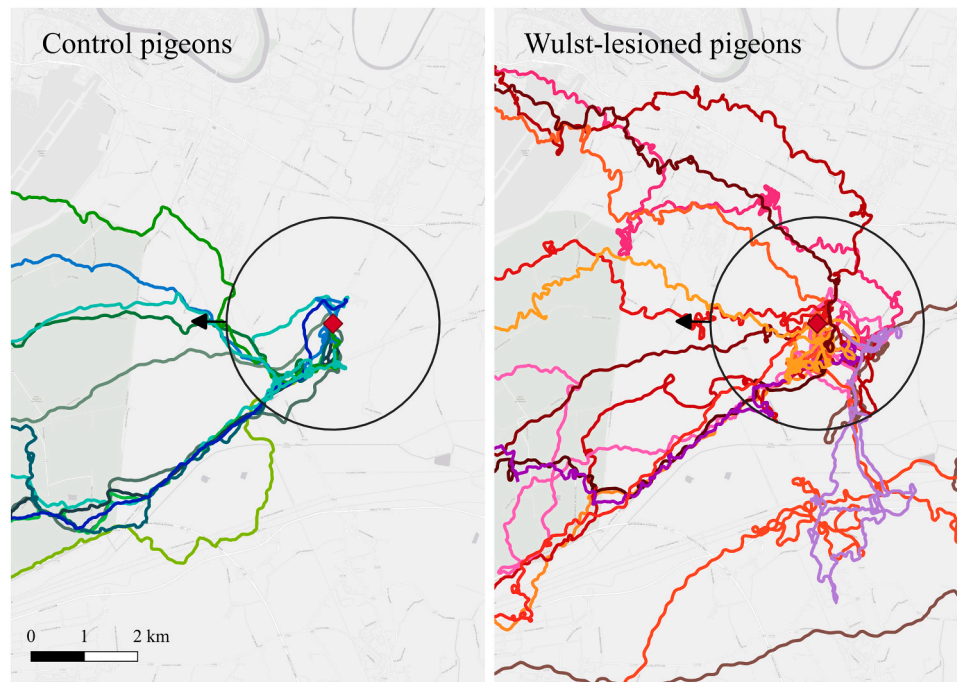
Fig. 2. First portions of the pigeons' flight paths corresponding to the decision making phase, the space within the black circles, during the first release from the Arnaccio (red diamond). The circle represents the 2 km boundary around the release point. The arrow outside the circle represents the home direction, which is at 271°. The control and the Wulst-lesioned pigeons tracks are shown in the left (from blue to green lines) and the right (from orange to purple), respectively.

(0.49–0.74); EImm, U = 66, p > 0.1, C 0.91 (0.70–0.95), W 0.78 (0.59–0.95); R_{EI}, U = 59.5, p > 0.1, C 0.86 (0.82–0.87), W 0.83 (0.75–0.86)).

3.3. Flight path efficiency: repeated releases across sites

Fourteen tracks of control birds and 13 tracks of Wulst-lesioned pigeons were excluded from the analysis because they flew home together with another bird. However, when birds joined only during one or two navigational phases, only the portions of a track relative to those phases were excluded from the analysis. This occurred for 1 and 5 tracks of control and Wulst-lesioned pigeons, respectively. Six tracks of control pigeons and 5 tracks of Wulst-lesioned pigeons were interrupted because the GPS battery ran out, or due to GPS malfunctioning. In these cases any incomplete phase was removed from the analysis (see Table S2, Supplementary material, for details). In the end, the flight paths of 9 Wulst-lesioned pigeons and 10 control birds were used in this analysis.

The general efficiency of the flight paths measured by EI scores, was significantly lower in the Wulst-lesioned pigeons compared to the control pigeons in each navigational phase (GLMM; DM-phase, EI estimated marginal mean ± se, C 0.62 ± 0.14, W 0.52 ± 0.15, p = 0.03; ER-phase, C 0.75 ± 0.10, W 0.68 ± 0.10, p = 0.009; LN-phase, C 0.76 ± 0.15, W 0.68 ± 0.16, p = 0.006; see Table 2 and Fig. 3). Interestingly, we found no differences in the low-spatial resolution tortuosity, as control and Wulst-lesioned pigeons displayed comparable EImm along the entire route home (GLMM; DM-phase, EImm estimated marginal mean ± se, C 0.82 ± 0.11, W 0.79 ± 0.12, p = 0.18; ER-phase, C 0.87 ± 0.11, W 0.84 ± 0.12, p = 0.12; LN-phase, C 0.85 ± 0.19, W 0.81 ± 0.20, p = 0.13; see Table 2 and Fig. 3). In contrast, a significant difference between Wulst-lesioned and control groups was found in high-spatial resolution tortuosity, emphasizing the small but frequent deviations of the flight path exhibited by the Wulst-lesioned pigeons (see Fig. 4). In fact, the R_{EI} of the Wulst-lesioned pigeons is smaller than that of the control group, indicating a higher level of oscillatory flight pattern in the Wulst-lesioned group (GLMM; DM-phase, R_{EI} estimated marginal mean ± se: C 0.74 ± 0.14, W 0.65 ± 0.15, p = 0.03; ER-phase, C 0.86 ± 0.09, W

0.80 ± 0.09, p = 0.002; LN-phase, C 0.89 ± 0.07, W 0.84 ± 0.8, p < 0.001; see Table 2, Fig. 3 and Figure S2, Supplementary material). The straightness of the path was also significantly affected by individuals' experience, irrespective of the treatment. During the DM and ER phases, the straightness of the path (measured by EI, EImm and R_{EI} indexes) increased with increasing experience (experience level, p < 0.001 for each index, see Table 2 and Figure S2A, S2B). However, in the LN phase, while both EI and EImm became greater across subsequent releases (experience level, p = 0.02 and p = 0.04 respectively, see Table 2), the oscillatory flight path (measured with R_{EI}) unvaried although individual experience increased (experience level, p > 0.5, see Table 2 and Figure S2C). Therefore, the difference in R_{EI} between control and Wulst-lesioned pigeons persisted across repeated releases.

A significant interaction between treatment and experience level was found only in the EI and EImm soon after release (DM phase, p = 0.04 for both parameters, Table 2). In particular, during the first three sets of releases, the control pigeons displayed significantly greater EI than Wulst-lesioned pigeons (post hoc: first set of releases EI C 0.50 ± 0.17, W 0.33 ± 0.19, p < 0.01; second EI C 0.55 ± 0.15, W 0.40 ± 0.16, p < 0.01; third EI C 0.60 ± 0.14, W 0.48 ± 0.15, p < 0.02) while no difference emerged in the next sets of releases. The EImm was significantly different between treatments only in the first two sets of releases (first set of releases C 0.73 ± 0.16, W 0.61 ± 0.17, p = 0.01; second C 0.77 ± 0.13, W 0.69 ± 0.13, p = 0.02) while no difference between treatments was found in the subsequent series of releases.

3.4. Individual route fidelity

The acquisition of route fidelity after leaving the release site up to the home area was comparable between Wulst-lesioned and control pigeons (GLMM; ER+LN phases combined, FF estimated marginal mean ± se, C 0.36 ± 0.13, W 0.31 ± 0.14, p = 0.30; Table 3 and Fig. 5). However, the tendency to retrace a previously flown flight path during the local navigation phase measured by the Spatial Occupancy Index turned out to be different between control and Wulst-lesioned pigeons. In fact, the Wulst-lesioned pigeons displayed significantly more scattering, i.e., less

Table 2

Results of the GLMMs applied on the EI, EImm and R_{EI} in each of the three homing navigational phases of the repeated releases. Phase: DM, decision making; ER, en-route; LN, local navigation. Significant results are shown in bold. DM, $N_{routeC} = 160$, $N_{routeW} = 130$; ER, $N_{routeC} = 161$, $N_{routeW} = 130$; LN, $N_{routeC} = 161$, $N_{routeW} = 128$.

Phase	Response variable	Factors	χ^2	df	p
DM	EI	treatment	4.44	1	**0.04**
		experience level	66.44	1	**<0.001**
		release site	6.41	2	**0.04**
		treatment x experience level	4.25	1	**0.04**
		treatment x release site	4.85	2	0.09
	EImm	treatment	1.77	1	0.18
		experience level	52.28	1	**<0.001**
		release site	0.03	2	0.98
		treatment x experience level	4.40	1	**0.04**
		treatment x release site	1.59	2	0.45
	R_{EI}	treatment	4.91	1	**0.03**
		experience level	44.32	1	**<0.001**
		release site	25.84	2	**<0.001**
		treatment x experience level	2.53	1	0.11
		treatment x release site	4.77	2	0.09
ER	EI	treatment	6.88	1	**0.009**
		experience level	67.23	1	**<0.001**
		release site	12.81	2	**0.002**
		treatment x experience level	1.94	1	0.16
		treatment x release site	0.44	2	0.80
	EImm	treatment	2.38	1	0.12
		experience level	56.49	1	**<0.001**
		release site	30.21	2	**<0.001**
		treatment x experience level	4.00	1	0.05
		treatment x release site	0.09	2	0.95
	R_{EI}	treatment	9.3	1	**0.002**
		experience level	33.97	1	**<0.001**
		release site	0.08	2	0.96
		treatment x experience level	0.02	1	0.97
		treatment x release site	4.79	2	0.09
LN	EI	treatment	7.50	1	**0.006**
		experience level	5.10	1	**0.02**
		treatment x experience level	0.13	2	0.72
	EImm	treatment	2.33	1	0.13
		experience level	4.19	1	**0.04**
		treatment x experience level	0.35	2	0.55
	R_{EI}	treatment	24.25	1	**<0.001**
		experience level	3.55	1	0.06
		treatment x experience level	0.003	2	0.96

fidelity, across their repeated flights compared to controls (Mann-Whitney test, U = 13, p < 0.05; C n = 10, SOI median (first and third quartiles) 0.64 (0.60–0.68), W n = 9, 0.75 (0.72–0.77); see Fig. 6).

3.5. Attraction to linear landmarks/landscape features

Examining the repeated releases from the three sites, Wulst-lesioned and control pigeons displayed a comparable tendency to follow linear landmarks during the DM phase of a homing flight (GLMM; DM-phase, LL estimated marginal mean ± se C 0.11 ± 0.22, W 0.10 ± 0.24, p = 0.27; see Table 4 and Fig. 7A). In contrast, a significant difference between the LL of the two groups emerged during both the en-route and local navigation phases. In fact, the Wulst-lesioned group showed a significantly lower tendency to fly along linear landmarks than the intact birds, a difference that persisted throughout the repeated releases from the three sites (GLMM; ER-phase, C 0.31 ± 0.11, W 0.23 ± 0.12, p

= 0.01; LN-phase, C 0.46 ± 0.16, W 0.33 ± 0.17, p < 0.001; Table 4, Figs. 7B and 7C). Moreover, in the LN phase, a significant interaction between treatment and experience level was found (p = 0.01). In particular, at the first release, the two treatments showed the same tendency to follow linear landmarks (post hoc: LL C 0.42 ± 0.19, W 0.40 ± 0.21, p > 0.05), while at the last release, a difference emerged. The control group increased the tendency to follow the leading lines, while for the Wulst-lesioned group, this tendency was reduced (post hoc: LL C 0.51 ± 0.19, W 0.27 ± 0.22, p < 0.001).

A difference in the tendency to follow linear landmarks from the different release sites was found both in the DM and ER phases. In the DM phase, pigeons were significantly more attracted to the linear landmarks from Arnaccio than from both La Costanza and Livorno (Tuckey test, Arnaccio vs La Costanza p < 0.001, Arnaccio vs Livorno p < 0.01; LL estimated marginal mean ± se, Arnaccio 0.35 ± 0.17, Livorno 0.23 ± 0.18; La Costanza 0.01 ± 0.33), and pigeons homing from Livorno were more attracted to linear elements than when released from La Costanza (p < 0.001). In the ER phase, pigeons were significantly more attracted to the linear landmarks from La Costanza than from Livorno (La Costanza vs Livorno p < 0.001; La Costanza 0.32 ± 0.10, Livorno 0.21 ± 0.11, Arnaccio 0.27 ± 0.11), and pigeons homing from Arnaccio were more attracted to linear elements than when released from Livorno (p = 0.02). Representative examples of control and Wulst-lesioned pigeons' tracks displaying different tendencies to follow linear landmarks are illustrated in Fig. 8.

3.6. Histology

The lesions sustained by the Wulst-lesioned pigeons were somewhat variable across subjects, particularly with respect to the lateral extent of the lesions (see Fig. 9 for the summary composite of the histological lesion reconstruction and Figure S3 for the individual histological recontructions). However, all sampled subjects sustained damage to the anterior hyperpallium apicale (A11.0 – A13.0 according to the pigeon brain atlas [39]) and to the hyperpallium densocellulare, the deepest lamina of the Wulst, particularly around A11.0. Irregular damage also occurred in the mesopallium around A11.0. We note that no detectable changes were observed in the motor ability of the pigeons (flight and walking behaviour) following the lesion.

4. Discussion

Consistent with what was reported by older experiments relying on the crude measures of vanishing bearings and homing performance [13], in the present work pigeons without a functional Wulst were unimpaired, compared to controls, at orienting homeward during the first release from each of the three sites and on the whole displayed the same homing success, although the Wulst-lesioned pigeons were significantly slower at homing from the two more distant release sites (Arnaccio and La Costanza). The lesion effect was at least in part due to the fact that, although displaying a comparable number of stops/kilometre, the Wulst-lesioned pigeons displayed longer "rest periods" compared to the intact pigeons during their first homing flight from Arnaccio and La Costanza. Therefore the impaired homing performance displayed by the Wulst-lesioned pigeons likely reflected at least in part a physical impairment, rather than a navigational deficit.

In contrast to hippocampal ablated pigeons [16,18], the Wulst-lesioned birds displayed an unimpaired ability to locate the loft within the home area even during their very first post-lesion release. Therefore, the pigeon Wulst does not seem to play a critical role in using visual-spatial cues to guide localising the loft within the home area. However, the analysis of the GPS tracking data revealed interesting effects of the Wulst lesions on the flight behaviour of the birds. In fact, the Wulst-lesioned pigeons showed flight paths consistently characterised by greater high-spatial resolution tortuosity, a reduced tendency to fly along linear (leading line) landscape features, and reduced tendency to
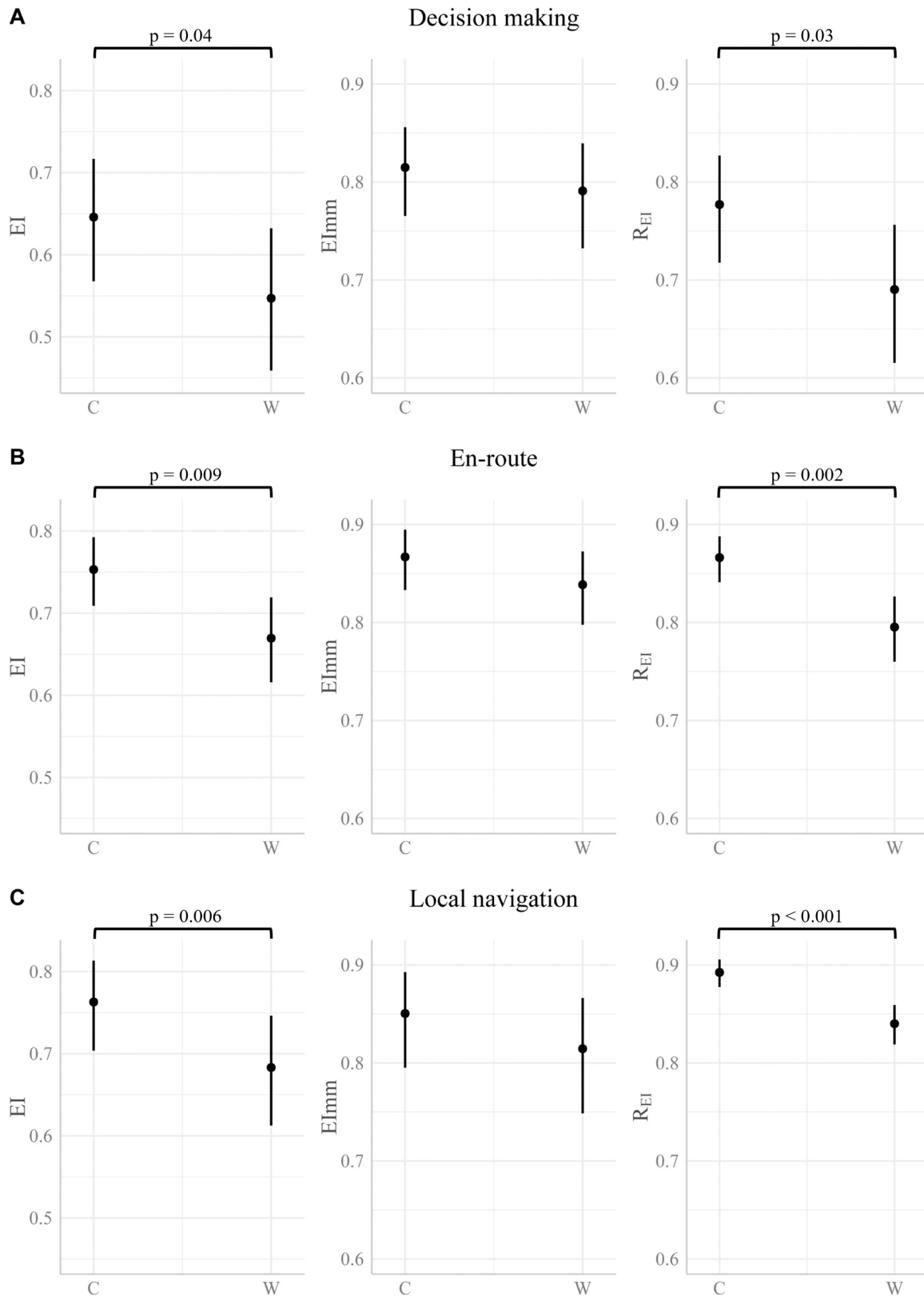
Fig. 3. Effects plots (Estimated mean ± 95% confidence intervals) of flight-path efficiency indexes during the three navigational phases of a homing flight. From left to right, estimated means of EI (overall path efficiency), EImm (a measure of the spatio-temporal low- spatial resolution tortuosity) and R_{EI} (a measure of spatio-temporal high-spatial resolution tortuosity, with smaller R_{EI} values corresponding to greater high-spatial resolution tortuosity, i.e. oscillation) of the control and Wulst-lesioned pigeons. Each row represents a navigational phase: A) decision making, B) en-route, C) local navigation. The significant p-values according to the Wald test on GLMMs results are reported in the plots.
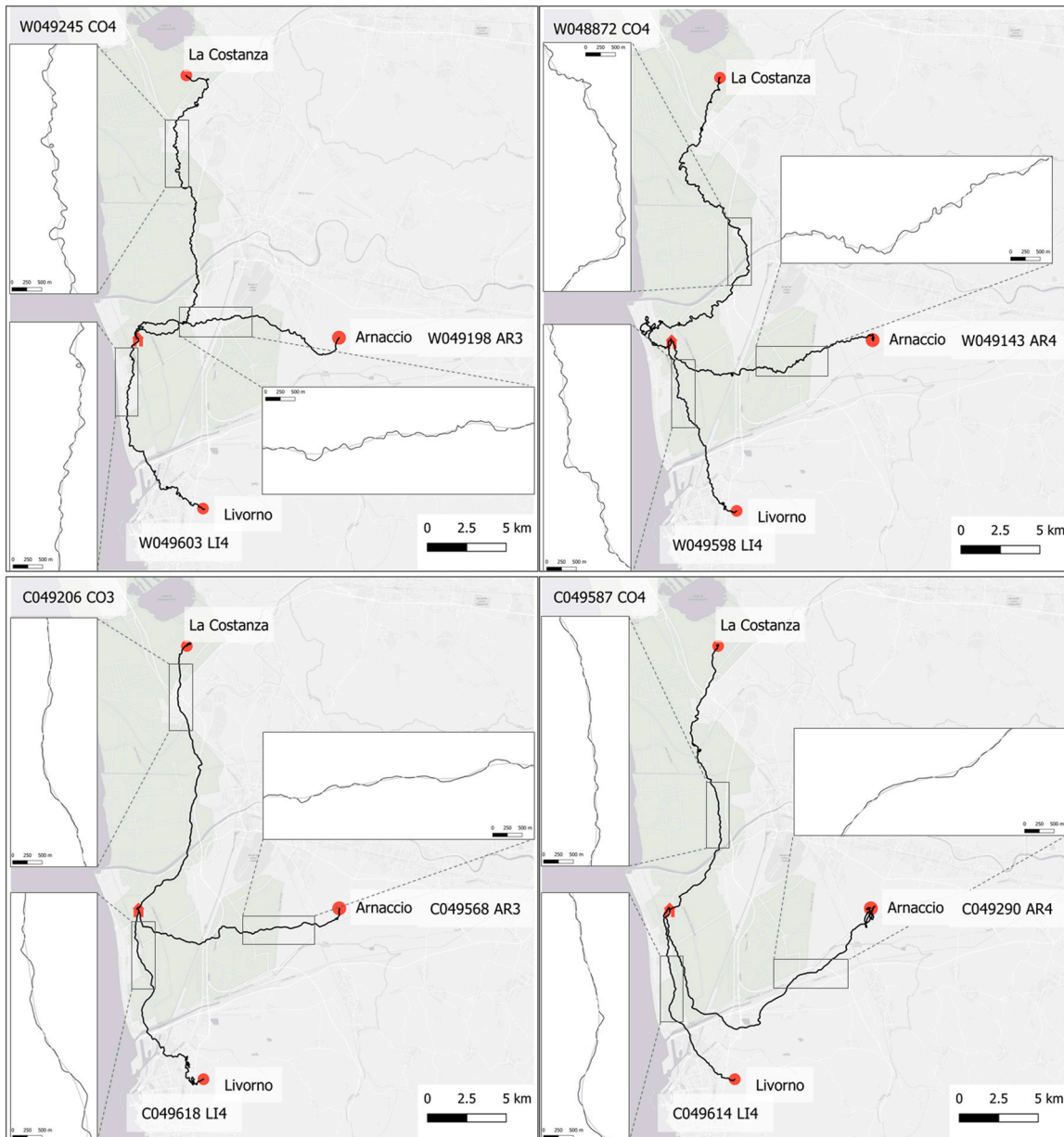
Fig. 4. High-spatial resolution tortuosity. Representative tracks of Wulst-lesioned and control pigeons displaying a different level of oscillation (measured by R_{EI}). In each panel three representative tracks from the three release sites are reported. The code next to each track indicates the following in order: treatment, subject identity, release site (AR, LI and CO refer to Arnaccio, Livorno and La Costanza, respectively) and experience level from each site. The black lines in both panels and zoomed boxes represent the original tracks. The gray lines in the zoomed boxes represent the tracks derived by the mobile mean of Latitude and Longitude across 40 fixes of the original tracks.

Table 3

Results from GLMM applied on FF (fidelity fixes) relative to the en-route (ER) and local navigation (LN) combined. Significant results are shown in bold. $N_{routeC} = 134$, $N_{routeW} = 107$.

Response variable	Phase	Factors	χ^2	df	p
% Fidelity Fixes	ER-LN	treatment	1.09	1	0.30
		experience level	152.79	1	**<0.001**
		release site	4.45	2	0.11
		treatment x experience level	0.26	1	0.61
		treatment x release site	2.31	2	0.32

retrace previous flight paths within the home area.

That little attention was paid by the Wulst-lesioned pigeons to landscape features is clearly illustrated by their initial orientation during the first release from Arnaccio, where conspicuous linear landscape features are present. In fact, when released from the Arnaccio release site, control birds consistently oriented south-westward, as they tended to follow several linear features running in parallel towards south-west (Arnaccio Road, Imperiale Channel, Chiara Ditch). When these parallel linear elements are seen from above they appear as an extended and conspicuous linear landmark, or leading line, that appears to attract intact pigeons [16]. In contrast, Wulst-lesioned pigeons did not show the directional bias observed in control birds and exhibited more variable directional choices. A very similar behaviour was observed in HF-lesioned pigeons on their first post-lesion release from Arnaccio [16]. Interestingly, a general diminished attention to linear landscape
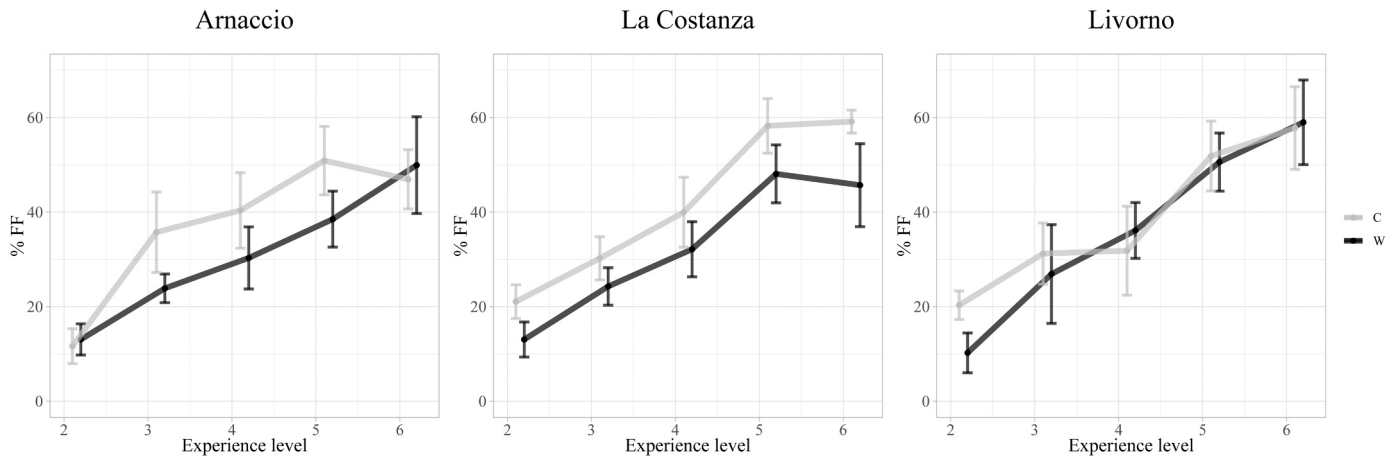
Fig. 5. Acquisition of route fidelity of control (grey) and Wulst-lesioned (black) pigeons during the combined en-route and local navigation homing phases. Mean percentage, with standard errors, of the FF scores (fidelity fixes, see 2.3.3 for further explanations) across the repeated releases (experience level) from each release site.
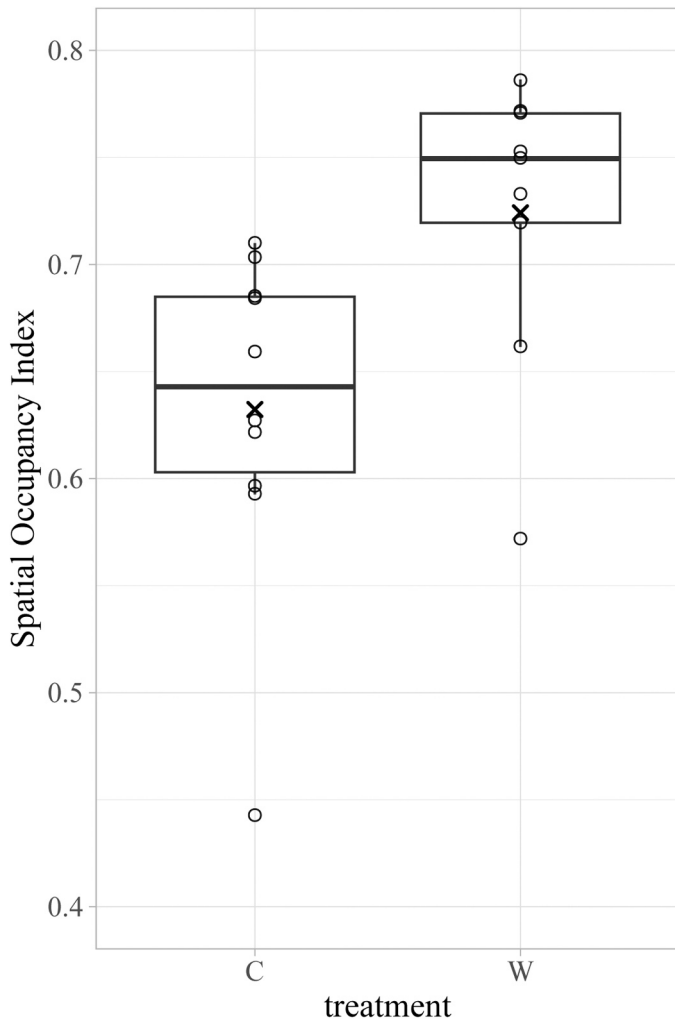

Fig. 6. Boxplots of the Spatial Occupancy Index (SOI) during the local navigation phase (see 2.3.3 for further explanations) in control and Wulst-lesioned pigeons (X symbols represent the mean, open circles represent individual data points).The smaller SOIs, the greater tracks overlapping. The p-value according to the Mann-Whitney U test is reported.

Table 4

Results of GLMM applied to LL (series of fixes aligned to linear landscape features). Phase: DM, decision making; ER, en-route; LN, local navigation. Significant results are shown in bold. DM, $N_{routeC} = 160$, $N_{routeW} = 130$, ER, $N_{routeC} = 161$, $N_{routeW} = 130$; LN, $N_{routeC} = 161$, $N_{routeW} = 128$.

Response variable	Phase	Factors	χ^2	df	p
% Fixes along Linear Landmarks	DM	treatment	1.20	1	0.27
		experience level	1.24	1	0.27
		release site	116.25	2	**<0.001**
		treatment x release site	4.10	2	0.13
		treatment x experience level	0.22	1	0.64
	ER	treatment	6.64	1	**0.01**
		experience level	6.84	1	**0.01**
		release site	25.58	2	**<0.001**
		treatment x release site	4.28	2	0.11
		treatment x experience level	0.55	1	0.46
	LN	treatment	12.16	1	**<0.001**
		experience level	0.02	1	0.89
		treatment x experience level	6.93	1	**0.01**

features was displayed by Wulst-lesioned pigeons throughout the whole experiment in comparison to control birds (see Fig. 8).

Unlike Arnaccio, the orientation of the Wulst-lesioned and control pigeons during the decision-making phase was similar during their first release from both Livorno and La Costanza. However, at their first release from La Costanza, both experimental groups displayed an initial bias towards the sea (west) and not towards home (southwest). Despite the unimpaired ability of the Wulst-lesioned pigeons to navigate home on their first release from each of the three sites, they displayed less efficient routes compared to control pigeons (see EI values in Table S1). This was due to both larger low- and high-spatial resolution tortuosity up to reaching the home area, i.e., the DM and ER phases (see EImm and R_{EI} values relative to the first set of releases in Table S1). Interestingly, also in the second set of releases the Wulst-lesioned pigeons displayed a greater low-resolution tortuosity (EImm) during the decision-making phase compared to the intact pigeons, perhaps reflecting a difficulty in learning the distinctive visual features of the release sites.

As stated above, the Wulst-lesioned pigeons displayed an unimpaired ability to localise the loft within the home area during the first release from each site. In fact, during those same, first three releases (one from each site) the Wulst-lesioned pigeons displayed comparable low-spatial
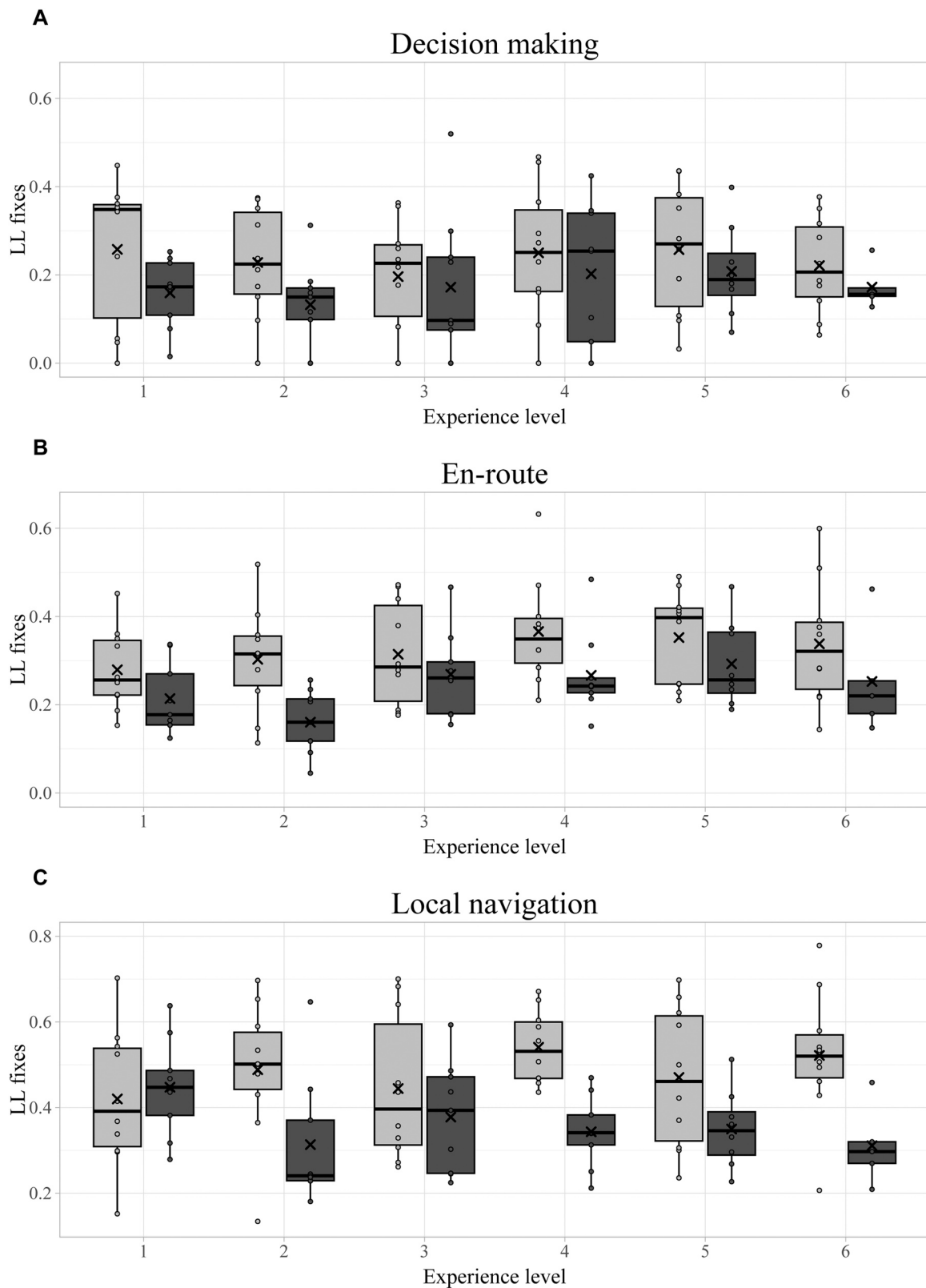
A

B

C

Fig. 7. Boxplots of the LL fixes (percentage of fixes aligned with a linear feature) distributions of the control (grey) and Wulst-lesioned (black) pigeons derived from the LL individual mean across the three releases from each site for each experience level. X symbols represent the second order mean, circles represent individual data points. Each panel represents a navigational phase: A) decision making, B) en-route, C) local navigation. See 3.5 for the statistical differences between control and Wulst-lesioned pigeons.
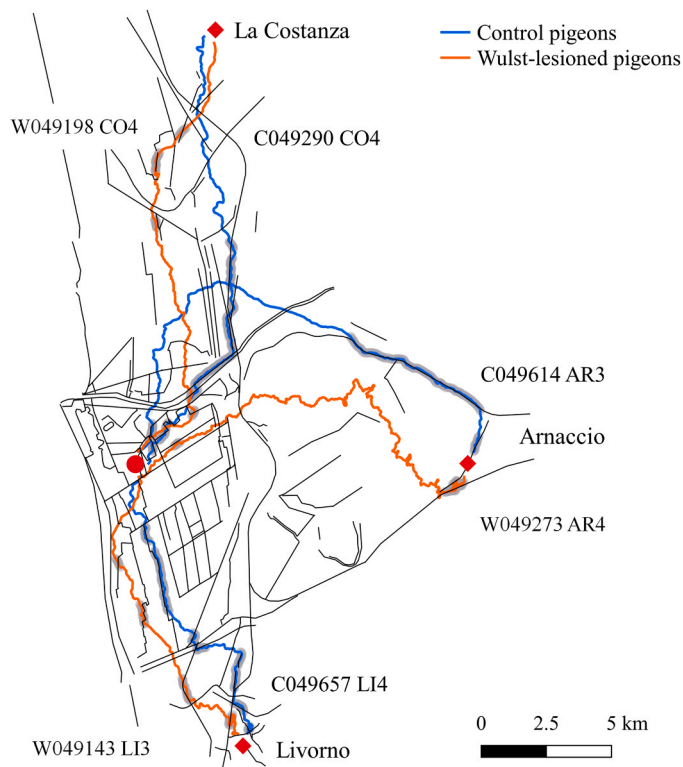
Fig. 8. Representative tracks of 3 Wulst-lesioned (red lines) and 3 control (blue lines) pigeons are shown. The black lines represent linear landmarks used in the analysis of LL fixes. The gray buffer around the tracks represents consecutive (more than 20 in a row) fixes closer than 100 m to a linear feature. The red diamonds and the red circle represent the release sites and home, respectively. Other explanations as in Fig. 4.

Fig. 9. Lesion reconstruction summary of the five sampled pigeons. The five reconstructions are superimposed so that the darkest gray areas indicate lesion damage common to all five pigeons, while the lightest gray areas indicate lesion damage observed in only one pigeon. E, Entopallium; HA, Hyperpallium apicale; HD, Hyperpallium densocellulare; M, Mesopallium. Coronal sections and associated anterior-posterior coordinates are derived from the pigeon brain atlas [39].

resolution tortuosity in the home area (LN phase) compared to the control birds. However, the Wulst-lesioned pigeons displayed a characteristic oscillating flight pattern (see below) in the last phase of the homing flight when challenged to localise the loft by using familiar visual landmarks. Because the birds in this experiment had pre-lesion homing experience, we can only say that the visual landmarks-based map learned and processed in part by the HF [16] was not affected by Wulst lesions. Whether an intact Wulst is needed for landmark-based map learning remains an open question.

Wulst-lesioned and control birds displayed comparable route fidelity acquisition after having left the release site and entering the area near the home loft (ER+LN phases). However, during the local navigation phase, Wulst-lesioned pigeons showed significantly higher Spatial Occupancy Indexes compared to the control pigeons, suggesting that when familiar visual landmarks become critical for locating the home loft, Wulst lesions affected the ability of the birds to consistently retrace previously flown flight paths across repeated releases. This might be a consequence of the diminished ability of the Wulst-lesioned pigeons to consistently fly along linear features of the landscape. In fact, it was previously observed that within the home area the acquisition of route fidelity in intact pigeons was largely dependent on their consistency in flying along the same linear landscape features, such as wood edges and roads [17]. The comparison of the flight path efficiency of control and Wulst-lesioned pigeons within the home area revealed no impaired ability of the latter in locating the loft within the home area. Therefore, Wulst-lesioned pigeons still seemed able to orient towards the loft using a behavioural strategy that does not require the consistent use of the same linear features of the landscape, differently to what previously observed for intact pigeons [17].

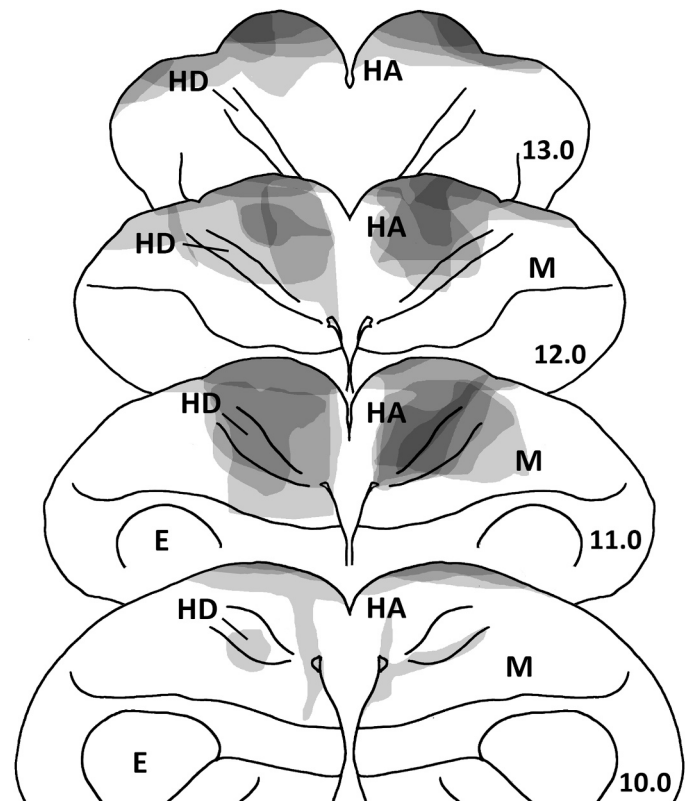In interpreting the role of the Wulst in familiar landmark-based

navigation it is important to consider possible similarities between the effects of Wulst lesions and the effects of HF lesions. In fact, two Wulst layers, the hyperpallium densocellulare (HD) and the hyperpallium apicale (HA) have connections to the HF [26,27], and similarities in the effects of Wulst and HF lesions might result from corrupted visual processing supported by Wulst-HF connectivity. Moreover, it is important to note that all our sampled subjects sustained damage to HA at A12.0 and A11.0 and to HD, the deepest lamina of the Wulst, at A11.0, which is a location where many neurons project to the HF and where there is a dense return projection from the HF [23,26,27].

One similarity between Wulst- and HF-lesioned pigeons suggested by our results concerns the persistent oscillatory flight paths displayed [17]. In fact, examining the tracks recorded during the repeated releases from the three sites, Wulst-lesioned pigeons consistently displayed an oscillatory, zig-zagging flight pattern, which, although gradually decreasing with experience, persisted throughout the whole experiment and remained consistently greater than that of control pigeons. We can only speculate on the function of the oscillatory flight paths occasionally seen in intact birds, especially during the first release from some sites, but consistently displayed by Wulst- and HF-lesioned pigeons [17] across repeated releases. One possibility is that the flight path oscillations, and the consequent decrease in the pigeon linear flight speed, might be important for processing landscape visual cues during the learning of a familiar landmark-based map. This would explain why intact pigeons with high level of experience for the overflown area display little consistent zigzagging of their flight paths. Following from this view, Wulst-lesioned pigeons displayed persistent oscillatory flight behaviour because they might require more time than controls to acquire/process a familiar landmark-based map. This would suggest the

existence of a cognitive-spatial brain network that encompasses both the visual Wulst and HF. The role of the Wulst in other cognitive processes has been reported in laboratory tasks, such as reversal learning [32,33]. We propose that visual input processed by the Wulst is likely shared with the HF, where visual information is integrated into a map-like spatial framework.

Laboratory studies have suggested the involvement of the Wulst in the processing of far field visual information [28–31]. Therefore, it is possible, perhaps likely, that the Wulst-lesioned pigeons had impaired perception of distant visual features and, therefore, a diminished linear speed might be needed for perceiving far field visual familiar landmarks. However, a debilitating visual impairment following Wulst lesions was unlikely, as Wulst-lesioned birds were observed to avoid obstacles (poles, telephone and power lines) in flight soon after release, and they were occasionally able to follow linear landmarks. Nevertheless, as mentioned above, following leading lines during a homing flight was consistently diminished in the Wulst-lesioned pigeons compared to the control pigeons. One might explain the diminished ability to consistently fly along linear features after Wulst lesions to be a consequence of impaired input from the lateral visual field [29,30]. However, the reduced tendency to fly along linear features displayed by the Wulst-lesioned birds does not seem to be related to an inability to see these elements at all. A previous homing experiment tested the homing behaviour of pigeons wearing translucent, frosted lenses preventing the view of landscape details. Because GPS technology was not available at the time, pigeons were radio-tracked [50]. This study showed that displaced pigeons wearing frosted lenses could fly homeward but were impaired in localising the loft within the home area. The lack of high acuity, detailed vision of the surroundings prevented those pigeons from finding their home loft [51]. In contrast, our study showed that Wulst-lesioned pigeons were able to locate the loft within the home area, and identified their own loft among others. This suggests that even if the lesions to the Wulst produced a form of short-sightedness, it would be certainly less severe than that observed in pigeons wearing frosted lenses. However, it must be considered that pigeons without a functional Wulst can still process visual cues with the Entopallium, which is the telencephalic projection field of the tectofugal visual pathway, that seems to process visual inputs from the frontal, near binocular visual field [21,24].

Interestingly, both HF- and Wulst-lesioned pigeons are less consistent in retracing previous routes during the last phase of a homing flight, as well as being challenged to localise the loft by relying on visual familiar landmarks [17]. In both HF- and Wulst-lesioned pigeons, the diminished route fidelity acquisition in the home area (LN) might be related to how leading lines, linear landscape features are processed. Although a direct comparison would be needed to carry out a formal statistical analysis, a difference seems to emerge between the effect of Wulst (present study) and HF [17] lesions in the context of local navigation. While Wulst lesions consistently resulted in a diminished tendency to fly along linear features compared to intact birds, HF-lesioned pigeons behaved as controls within the home area in terms of the number of fixes aligned along linear features. However, while intact birds consistently flew along the same linear features during subsequent releases, HF-lesioned pigeons were unable to develop route fidelity by incorporating linear features in a map of familiar landmarks. In summary, in a very familiar area, such as the area near the loft where visual landmarks become critical for homing, intact pigeons display a robust route fidelity by following the same linear features during subsequent releases. In contrast, Wulst-lesioned pigeons seem to have difficulty generally flying along linear features while HF-lesioned pigeons display an unimpaired ability to fly along linear features, but impaired fidelity to/memory of paths guided by linear-landmarks.

The current study leaves open the question of what effect the Wulst lesions may have had on pigeons released from some distant, unfamiliar site, i.e., navigation based on their olfactory map. Implicit in our narrative is that the Wulst and hippocampal formation collaborate in their support of visually-guided navigation. Given that the hippocampus plays no necessary role in supporting navigation form distant, unfamiliar locations [20], it follows that Wulst lesions should have little effect as long Wulst lesions do not compromise seeing the sun, and there is no evidence for such an effect. Also, covering the eyes with translucent frosted lenses does not substantially compromise the ability of homing pigeons to fly off in the home direction from distant, unfamiliar sites [50]. Therefore, the prediction would be that Wulst lesions would have little impact on navigation where guidance by familiar, visual landmarks is unimportant, but only by carrying out releases from distant, unfamiliar locations can this predicition be tested.

In conclusion, our working hypothesis is that an intact Wulst is involved in attention to and fast memorization of visual landscape features, and linear leading lines in particular. The persistent oscillating flight paths might reflect a difficulty in remembering detailed features of the landscape. However, it is not clear whether this is due to a cognitive impairment in processing visual cues or, alternatively, to an impaired perception of visual features. Further studies are needed to unravel the functional contributions of the hippocampal formation and Wulst in the brain network that supports the learning/implementation of the homing pigeons' familiar landmark-based map. For example, Budzynski et al. [28] used a regression analysis to reveal that deeper layers of the Wulst, e.g., HD, are more important in support of sun compass-based associative learning when pigeons are tested in an experimental arena, and it is also HD that shows strong reciprocal connections to the hippocampal formation [27]. One important goal of any future research would be to carry out targeted Wulst lesions, relying on electrolytic lesion procedures, to better understand the relative contributions of the different Wulst subvisions in support of homing pigeon navigation.

CRediT authorship contribution statement

Verner P. Bingman: Writing – original draft, Investigation, Conceptualization. **Anna Gagliardo:** Writing – original draft, Investigation, Formal analysis, Conceptualization. **Sara Cioccarelli:** Writing – original draft, Visualization, Investigation, Formal analysis. **Dimitri Giunchi:** Formal analysis, Conceptualization. **Enrica Pollonara:** Visualization, Investigation. **Giovanni Casini:** Visualization, Investigation.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bbr.2024.114971.

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