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Navigational maps in homing pigeons: GPS-tracking experiments on the role of the stimuli involved in pigeons' navigation

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1. GENERAL INTRODUCTION

1.1 THE NAVIGATIONAL SYSTEM OF HOMING PIGEONS

The migratory movements of animals and birds in particular, have always aroused fascination and scientific interest. Within the animal kingdom, birds are unrivalled at covering large distances quickly and passing over geographic barriers during their migratory journeys. Migratory commuters movements make the birds optimal experimental models used in the study of the mechanisms underlying navigation. Besides, birds are able to move towards specific sites where they can find suitable ecological conditions for feeding, roosting, mating and nesting. This extraordinary spatial ability requires birds to rely on navigational mechanisms allowing localization of even small targets. The ability of an animal to come back to its home range or foraging areas can be considered a special case of navigation and it is often called “homing” (Papi, 1992b). In the literature it is called “true navigation” the ability of an animal to return to its original location after displacement to a site in unfamiliar territory, without access to familiar landmarks, goal emanating cues, or information about the displacement route (Able, 2001; Schone, 1984). In migratory birds this particular ability is developed and shaped by experience, as juveniles at their first migration are unable to perform true navigation (Perdeck, 1958; Thorup et al., 2007). In fact, young birds at their first migration seem to perform a “vector navigation”, that is the ability of the birds to maintain a particular orientation for a specified time or distance (Bingman and Cheng, 2005). By contrast, some displacement experiments during fall migration have demonstrated that adults navigate, as they fly in the direction that will take them to their normal wintering grounds.

One of the key issues in the study of navigation is to understand the nature of the cues used by animals to establish their position relative to the goal and the nature of the cues used to determine and maintain a specific direction during their movement. These two distinct phases have been called by Kramer “map step” and “compass step”, respectively (Kramer, 1961). As regards the compass step, it’s known that birds posses at least two compass mechanisms, based on the geomagnetic information (Merkel and Wiltschko, 1965) or based on the sun information (Kramer, 1953) (see chapter 1.3).

Before to investigate further aspects of birds’ orientation, it is appropriate to make a brief overview on the development of knowledge about the subject of this study, the homing pigeon (*Columba livia*), in order to understand why the knowledge of navigational mechanisms used by these animals can be useful for understanding the mechanisms of homing in birds.

1.2 HOMING PIGEON LIKE A MODEL

Homing pigeons are a domesticated breed of the wild species *Columba livia* (Gmelin 1789), which probably started to be selected by humans around 3000 A.C. following the first crops of graminaceous plants in the eastern Mediterranean, on the basis of their homing ability. The importance of this breed in the study of navigation is testified by the fact that the present knowledge about the mechanisms used by the birds to find their way home from remote areas is largely due to experiments conducted mainly with homing pigeons. There are many reasons that have elected the homing pigeons as experimental model. Hundreds of pigeons can be easily kept in lofts and used throughout the whole year. Their motivation to return to their loft is not limited to the breeding season. Their size is neither too small nor too large: on one hand, they are large enough to be observed with binoculars over approximately 2 Km and to carry the weight of a small data logger to record their flight paths; on the other hand they are small enough to be easily handled and transported. At first glance, it might appear inappropriate to use a non migratory bird, selected by humans, as model for the study of wild birds' navigation. However, closely related species (e.g. *Columba palumbus*) do migrate, and Berthold et al. (1990) have shown that offspring of partially migratory birds of a given species can be genetically transformed, within only few generations, by means of selective breeding to either full migrants or non-migrants. Although the rock pigeon is usually a sedentary pigeons, the navigation mechanism detected in homing pigeons might indicate mechanisms used by other birds during migration. In fact, it seems extremely unlikely that, during the domestication of the rock pigeon (*Columba livia,* Gmelin), a completely new navigational mechanism that did not exist before in the genus *Columba*, has been implanted into the strains of homing pigeons.

1.3 THE COMPASS MECHANISMS

The compass mechanism refers to the ability of an animal to orientate in a particular compass direction, without relying on landmarks. Birds make use of compasses during migration and homing. The compass systems used by homing pigeons are the sun compass and the magnetic compass, which is particularly needed to orient in overcast conditions.

1.3.1 The sun compass

In the 1953 Kramer observed that starlings (*Sturnus vulgaris*) housed in a cage showed directed migratory restless, using the sun to obtain directional information (Kramer, 1953). To use the sun as a compass for direction determination, an animal must know the current sun's azimuth and the corresponding time of the day. This process is regulated by an endogenous rhythm or biological clock. Therefore the use of the sun as compass reference is based on the birds' ability to compensate for the azimuth change during the day by varying their angle of orientation with the sun. The best evidence that an animal relies on a sun compass is obtained by subjecting it to a shifted light-dark cycle and observing a predictable deviation in orientation. Schmidt-Koenig (1958; 1961) found that the initial bearings of pigeons, that have been living for several days in a light-dark cycle shifted by 6 h either forward or backward, deviated from those of non-shifted controls in a predictable way (by roughly 90° counterclockwise or clockwise, respectively). In fact, the phase-shift treatment induce a deflection of the initial orientation of the birds approximately corresponding to the difference between the sun azimuths at the real and the subjective time (Fig. 1). However, in some experiments, the initial orientation of pigeons subjected to a clock-shift treatment showed a deviation lower than expected. This result may be due to some orienting factors inducing a correction of the directional error. In the next chapters we will discuss about the nature of the information that might affect the size of deflection in orientation following clock shift.

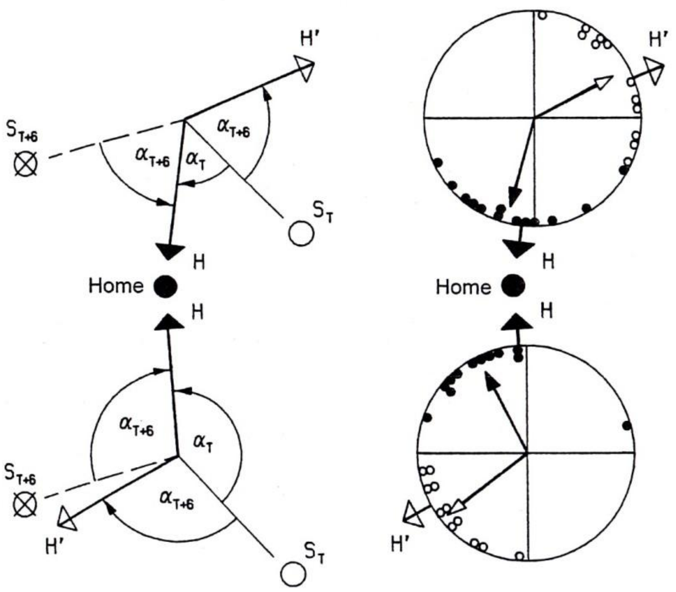

Fig. 1. The clock-shifted birds (open circle) were releases, alternately with unshifted controls (filled circles), at two sites about 30 Km North and South of home. Left-hand diagrams show the theoretically expected flight directions on the assumption of use of a sun-azimuth compass. If the birds, at time T (here 10:00 A.M.), select an angle αT relative to the sun ST , they fly in the direction toward home H. At the time T, the shifted clock of the birds subjected to the shift treatment, shows T+6h. At that time (4:00 P.M.) the sun would be at position ST+6; H would be reached by selecting an angle αT+6 . By keeping this angle relative to the actually visible sun ST, the birds would achieve the course H'. Under the given conditions, H' is approximately 120° left from H. Right-hand diagrams show actually observed vanishing bearings with their mean vectors. The experimental pigeons were as well oriented towards H' as the controls were towards H (Wallraff, 1988a).

1.3.2 The magnetic compass

Even though the use of visual cues help to determine a compass direction, what happens if the sun is not visible, for example, under an overcast sky? Even under these conditions, pigeons can navigate without problems, which indicate that pigeons must be able to use an additional compass based on geomagnetic information (Keeton, 1969).

It is well known that the Earth is a huge magnetic dipole with its poles located near the rotational poles (Fig. 2). The axis of the magnetic field is tipped with respect to the rotation axis of the Earth. Thus, true North (defined by the direction to the North rotational pole) does not coincide with magnetic North (defined by the direction to the North magnetic pole); as a consequence, the compass directions must be corrected by fixed amounts at given points on the surface of the Earth to yield true directions. The field lines defining the structure of the magnetic field are similar to those of a simple bar magnet, leave the Earth's surface from the magnetic South pole, curve around the Earth's surface before falling to the magnetic North pole. The inclination angle at which the magnetic field lines intersect the Earth’s field varies predictably with latitude. The magnetic field is a vector field characterized by a vector function of the observation point and time, indicated as “*total intensity of the field*”.

The intensity of the geomagnetic field is maximum at the magnetic poles where the lines are oriented vertically with respect to the Earth’s surface (~63000 nT) and minimum near the magnetic equator (~23000 nT), where the field lines are parallel to the Earth's surface. So there is a gradient ranging from the magnetic poles to the equator in both hemispheres of the Earth.

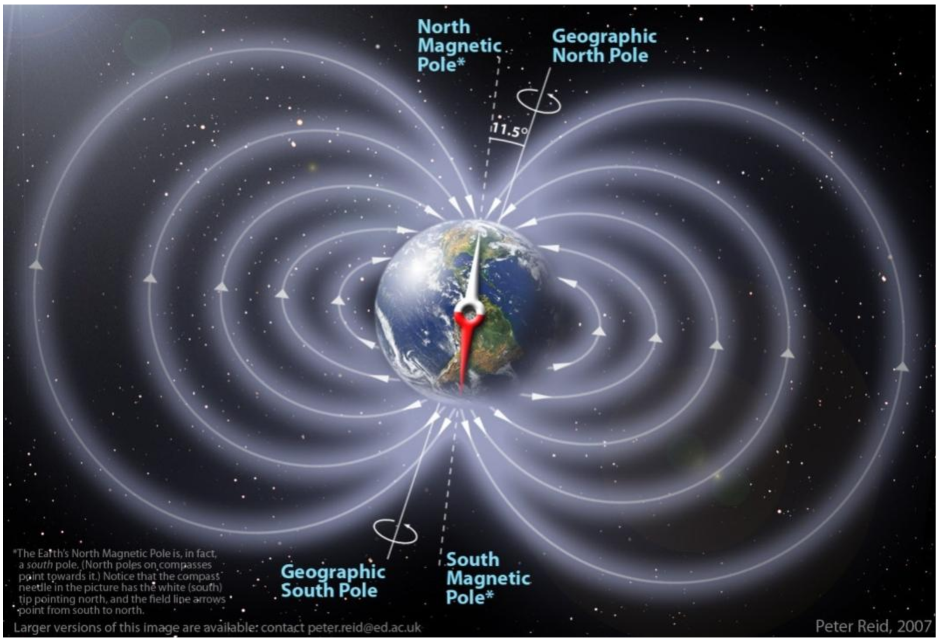

Fig. 2. Representation of the Earth’s magnetic field.

The angle on the horizontal plane, between the direction of the geographical North and the magnetic North is called **magnetic declination**, and it is just a measure of the error that performs our compass pointing the magnetic North compared to the geographical North.

The angle between the magnetic vector and the horizontal plane is the so called magnetic inclination, which is negative in the northern magnetic hemisphere and positive in the southern magnetic hemisphere. If we consider the vector direction, in the northern hemisphere it will point down (the slope is negative); vice versa in the southern hemisphere the vector pointing upward until it becomes vertical to the southern magnetic pole. Therefore each point of the earth's magnetic field is represented by a vector, whose angle and length (intensity) varies in different parts of the Earth's surface. This field can be locally altered by some materials forming the Earth's crust, creating local areas of magnetic anomaly with increases or decreases of the magnetic intensity, although it was suggested that these variations are insignificant when compared to the regularities of the field.

According to some authors, the information of magnetic origins can constitute parameters characterized by such reliability to have allowed the evolution of magnetic navigation systems in different organisms (Papi et al., 1971). It has been proposed that the directional information from the Earth's magnetic field could be used by pigeons both in the compass step than during the orientation and navigation in the map step (Wiltschko and Wiltschko, 2006; Walcott et al., 1988).

In pigeons, the existence of a magnetic compass has been first demonstrated by Keeton in 1971 by attacking on the pigeons backs a bar magnet (Keeton, 1971). This treatment affected the pigeon's initial orientation only under overcast sky, and not under sunny conditions. Overall, several experiments have been conducted by releasing pigeons in overcast sky with permanent magnets attached to their wings, heads and/or beaks (Ioalè, 1984; 2000; Wallraff, 1986; Moore, 1988). Taken together these experiments "seem to provide evidence that under certain conditions the magnets can confuse homing pigeons" (Keeton, 1971). At the same experimental circumstances but in sunny conditions, the effect of the bar magnets on the initial orientation of the pigeons was not reported. This has been interpreted as an evidence that pigeons preferentially use the sun compass to establish the home direction, but that they possess a second compass system based on the geomagnetic information, used when the sun information are not available (Kramer and Riese, 1952; Schmidt-Koenig, 1991).

During experiments conducted in laboratory, it has been demonstrated that the birds' magnetic compass is an 'inclination compass' (Wiltschko and Wiltschko, 1972; Wiltschko, 1968). A group of European robins (*Erithacus rubecula*) were subjected to the rotation of the magnetic North through a system of Helmholtz coils; as a consequence birds modified their migratory direction orienting with respect to the "new" magnetic North (Wiltschko, 1968). The robins were therefore not able to distinguish between the magnetic North and magnetic South, but between direction "toward the pole" and direction "toward the equator".

It has been suggested that in contrast to the sun compass, which is learned, the magnetic compass is innate and does not depend on experience and learning (Wiltschko and Guinner, 1974).

In the 2001 it has been hypothesized that the two compass systems are active simultaneously during homing (Wiltschko and Wiltschko, 2001c). Therefore, in the Wiltschko hypothesis (1994a; 2001b) the contemporary use of the sun and magnetic compass would be responsible of the reduction of deviation observed in clock-shift experiments from familiar locations. This hypothesis is supported by the results obtained in a clock-shift experiment during which shifted pigeons magnetically treated, showed larger deflection with respect to shifted pigeons without additional manipulation. The authors interpreted these results as a demonstration that the pigeons use simultaneously both compass mechanisms. Gagliardo and colleagues confirmed these results with a GPS study (Gagliardo et al., 2009c).

A candidate magnetoreceptor involved in the magnetic compass mechanism is the retina. According to the radical pair model the reception mechanism involves photosensitive proteins that form radical pairs when excited by light. This reaction is modulated by the magnetic field and would differentially occur in different parts of the retina (Ritz et al., 2000). This process would lead to perceive the magnetic field as a visual pattern and would mediate the magnetic compass mechanism (Ritz et al., 2000; Wang et al., 2006). The cryptochromes contained in the retinic ganglionar cells are the photosensitive proteins presumably involved in this process and the Cluster N, a region of the visual system partly coincident with the Wulst, has been suggested as the brain portion processing the magnetic stimuli (Heyers et al., 2007). The experimental evidences yielded so far show an increased expression of the cryptochromes at night in a migratory passerine displaying an oriented locomotory activity on the basis of magnetic cues; such increase was not observed in a non migratory species (Mouritsen et al., 2004).

1.4 THE NAVIGATIONAL MAP MECHANISMS

The compass direction systems alone cannot provide the information about the position of an animal in relation to its goal. Therefore a second mechanism is needed: the navigational map.

The position finding mechanism of an animal with respect to the goal is based on information collected from local environmental cues. On the basis of the fundamental differences in the information upon which they might be based and their mode of operation, it is possible to distinguish between two kind of maps: the *mosaic* map and the *gradient* map (Wallraff, 1988b) (Fig. 3). The *mosaic* map model assumes that the area, in which the map is functional, is fragmented into a number of zones. The spatial relationships are learned by the animal during direct explorations or exploiting other source of information (see olfactory map hypothesis below) (Wiltschko and Wiltschko, 1978; Wallraff et al., 1994). A g*radient* or *grid* map (Able, 2001; Wallraff, 2005b) assumes instead the presence on at least two gradients of any physical substrate, relatively stable over time and space, which vary systematically over sufficiently large regions. Assuming that these gradients extend monotonically beyond the familiar area, a displaced bird could obtain an estimate of its position relative to home by comparing the remembered values of the variables at home, with the values at the release site. In theory, the range of such a map is unlimited, in practice its range and accuracy would depend on the spatial extent of the gradient fields and their uniformity.

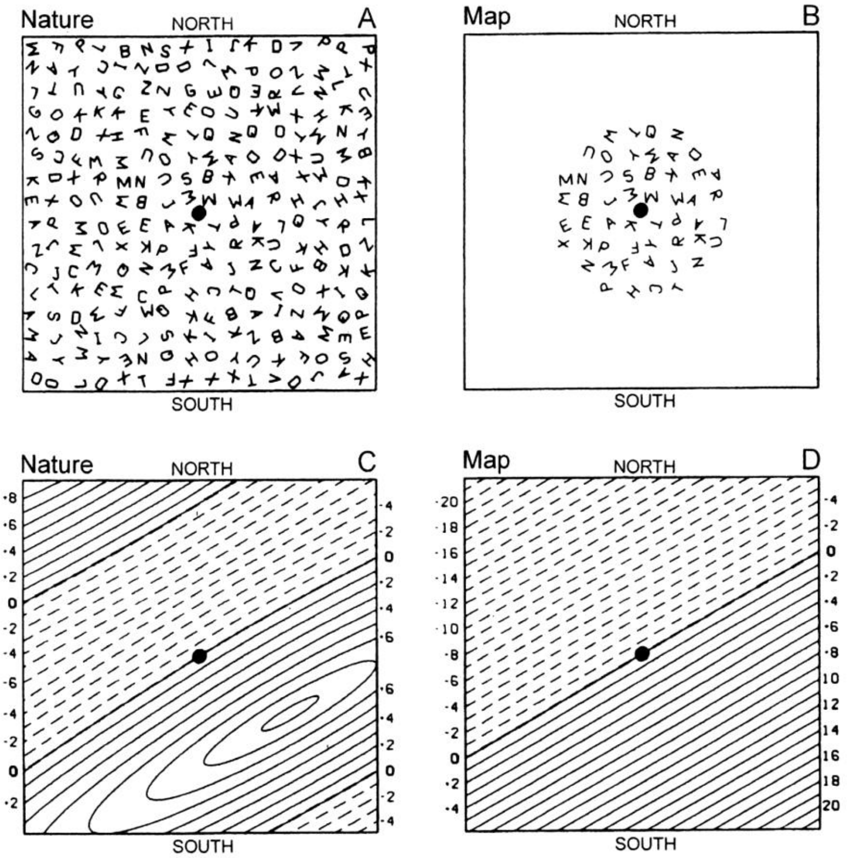

Fig. 3. A mosaic of landmarks, symbolized by *letters*, surrounding a bird's home site (central dot). B. The corresponding mosaic map (topographical map), which is limited in extent by the bird's range of experience. C. Isolines (arbitrary units) of a fictitious gradient field. The *line* representing the scalar value observed at home is definite as 0, higher values are indicated by solid and lower values by broken lines. D. The bird's corresponding gradient map as established by extrapolation of home-area conditions. Note that in C and D, for simplicity, gradients of only one variable are shown. For complete site localization, at least two gradient fields are required intersecting at sufficiently large angles. The sections shown are thought to be of different size; side of the square in A and B may be at most few hundred kilometres, those in C and D 1.000 Km or more. (Wallraff, 2005a)

1.4.1 Navigation over unfamiliar areas

When displaced at an unfamiliar release site distant from home, pigeons do not have visual contact with the home area and therefore must rely on a navigation map mechanism to determine the home direction. While there is a general agreement on the nature of the cues used by birds to take a direction in space, the nature of the information used by birds to determine their position with respect to the goal after a displacement to unfamiliar locations is one of the most debated issues in animal navigation. Nowadays, two competing hypotheses are subjects of a lively scientific debate: the existence of a map based on olfactory stimuli (Papi et al., 1972; Wallraff, 2005a) or the use of a map based on geomagnetic information (Walcott, 1982; Wiltschko and Wiltschko, 1995; 1998).

1.4.1.1 The Olfactory navigational map

Papi and colleagues discovered the importance of olfactory cues for bird navigation in 1971 (Papi et al., 1971). This research group conducted an experiment during which pigeons with the olfactory nerves sectioned and false-operated pigeons were released from an unfamiliar location. The authors observed that the birds subjected to the section of the olfactory nerves were disoriented and impaired in homing in comparison to the control false-operated birds. Nevertheless, these results could be ascribed to a non-specific effect of the surgery. In a second study, Papi and collaborators (1972) subjected the pigeons to two treatments: unilateral nerve section and occlusion of the ipsi- and/or contralateral nostril. The nostril plug was placed ipsilateral to the lesion for the control pigeons and contralaterally for the experimental birds. In this way, only the control pigeons had access to the odour information, but both groups had the same disturbance. Pigeons which could smell with one nostril (unilateral sensory deprivation), performed significantly better than the birds completely deprived of olfactory cues (bilateral sensory deprivation). These findings showed that the poor navigation performance in the first experiments was effectively caused by olfactory deprivation and not by the invasiveness of the surgery (Papi et al., 1972).

On the basis of these results, Papi formulated the *olfactory navigation hypothesis*. According to this hypothesis, pigeons during their first months of life learn the associations between the environmental odours carried by the winds and the direction from which the winds blow. Once at the unfamiliar distant release site, the pigeons recognize the local odour profile, compare it to the wind direction associated with that odour profile experienced at the loft and thus determine the direction of displacement (Papi et al., 1971; 1972; 1973; Ioalè et al., 1990). The olfactory navigation hypothesis provides therefore evidence that the pigeons build up a mental representation of the environmental odours distribution of the surroundings.

The olfactory navigation hypothesis has been experimentally examined with two different kinds of manipulation: i) manipulation of the olfactory apparatus; ii) manipulation of the olfactory information at the home loft, during the transportation or at the release site (Papi, 1991; Wallraff, 2005a; 2004).

A systematic and specific impairment of the homing performance in both naïve and experienced pigeons released from unfamiliar locations has been observed in many experiments of manipulation of the olfactory system. Among these, there are experiments of olfactory deprivation achieved with different methods: section of the olfactory nerves (Papi et al., 1971), occlusion of the nostrils, insertion of small tubes of silicone from the nostrils to the choanae to prevent the contact between the olfactory mucosa and the inspired air (Keeton et al., 1977), anaesthesia of the olfactory epithelium (Schmidt-Koenig and Phillips, 1978), washing the olfactory mucosa with zinc sulphate which induce necrosis of the olfactory epithelium (Benvenuti et al., 1992). Olfactory deprivation experiments have been conducted in different countries of the world producing the same results, that anosmic pigeons are impaired in navigation (Benvenuti et al., 1998; Wallraff, 2005a). In addition to the olfactory mucosa manipulations, experiments of lesions to brain regions involved in the elaboration of olfactory stimuli were also conducted. Bilateral lesions of the piriform cortex, which receive the main projections from the olfactory bulbs and it is thought to process the olfactory information in birds (Reiner and Karten, 1985; Bingman et al., 1994), also produce dramatic navigational deficit in the pigeons (Papi and Casini, 1990; Gagliardo et al., 1997).

The manipulation of olfactory information at the home loft can be done, either manipulating the direction of the winds carrying natural environmental odours, or providing artificial wind born odours. In the first case, three main types of wind manipulations have been employed: (1) shielding the natural winds; (2) deflecting the winds clockwise or counter-clockwise; (3) reversing the wind directions (Papi, 1990; Wallraff, 2005a). In experiments conducted with birds raised in screened aviaries, pigeons could see the surrounding landscape, but were not able to associate the odours carried by the winds with the wind directions, because the air entered from the roof of the aviary. The navigational performances of these pigeons were impaired, while the pigeons confined in aviaries open to winds were homeward oriented, with homing performances better than the experimental groups (Wallraff, 1966; 1979; Gagliardo et al., 2001a; Odetti et al., 2003). Following the deflection of the winds, pigeons released at unfamiliar sites displayed a corresponding deflection of their initial orientation, indicating that the navigational map had been rotated. In an elegant experiment pigeons were confined in aisle-shaped aviaries. The longest sides were screened to the wind and the shorter sides were equipped with large ventilators. For the experimental groups, the ventilators were turned on when the wind blew from the opposite direction, resulting in a reversal of the dominants winds' direction. Once released, the experimental pigeons showed a reversed orientation with respect to that displayed by the control pigeons exposed to air fluxes with direction coherent with the natural winds (Baldaccini et al., 1975; Ioalè et al., 1978). These results confirmed the key role played by the winds for the development of the navigational map. Interestingly birds raised confined in screened aviaries could not develop a navigational map *ex novo* in adulthood even after months of exposition to the natural winds, suggesting a sensitive learning period during the first 2-4 months after fledging (Gagliardo et al., 2001a). However if the birds are allowed to perform spontaneous flights around the loft in adulthood, are able to improve their navigational performances (Odetti et al., 2003).

The specific role of environmental odours in pigeon navigation is supported by experiments in which the birds were exposed to air currents carrying artificial odours. These pigeons were then exposed to the same odours at the release site. Regardless the home direction, they oriented in the direction opposite to that of the odour air current they were exposed to in the aviary. (Ioalè et al., 1990).

During a creative experiment Papi and colleagues (1974) confined two groups of pigeons in an aviary fenced with plastic and bamboo material. Each group were subjected to artificial odours: one of the two groups was subjected to an odours wind of olive oils from South and an odours wind of a solvents mixture from North, the other group underwent the opposite treatment. Each of the two groups of pigeons occupied half of the aviary and each had its own corridor. The direction taken by the birds after displacement was consistent with the direction of the odours perceived by pigeons in the aviary, showing that the treatments with odours air currents influence the initial orientation in a predictable way. The birds showed the tendency to fly in a direction opposite to that from which they were accustomed to perceiving the same odour when subjected to wind treatments in the aviary (Papi et al., 1974).

With another kind of protocol the pigeons can be fooled about the olfactory information gathered at the release site. Pigeons transported in pure air, were exposed to olfactory information of a false release site and then released, after anaesthesia of the olfactory mucosa, at a release site located in the opposite direction with respect to home. The control birds were exposed to the olfactory information of the true release site. The initial orientation of the birds was consistent with the direction of the false release site, where the pigeons perceived the local olfactory information. (Benvenuti and Wallraff, 1985; Kiepenheuer, 1985; Dall'Antonia et al., 1999). To determine the direction of displacement pigeons rely also on the information perceived during transportation. This has been demonstrated by experiments of displacement with divergent pathways (detour experiments). In these studies, pigeons transported at the release site through two different ways showed, upon release, a tendency to orient in the opposite direction with respect to the initial tract of the journey towards the release site (Papi et al., 1978; 1984).

As concerning the inner structure of the olfactory map, two non-mutually exclusive models have been proposed. According to the hypothesis of a mosaic map proposed by Papi and collaborators, chemical compounds would provide site-specific information patching the territory into sub-regions characterized by specific scents (Papi et al., 1972). According to a gradient map proposed by Wallraff, gradients of chemical compounds in the atmosphere provide positional information useful for the pigeons to determine their position with respect to home (Wallraff, 2000). In fact, sampling the air in large areas of Germany, Wallraff showed that hydrocarbons of artificial origin have a distribution sufficiently stable in space and time, to form the basis of a navigational map (Wallraff and Andreae, 2000). However it is still unclear what are the substances involved in the map mechanism of pigeons, and as regarding the hypothesized models, both could allow the pigeons to determine the direction of displacement rather than the exact location of the release site.

Since the olfactory navigation hypothesis predicts that environmental odours perceived at the release site provide information only about the direction of displacement, it has been hypothesised that once within the home area, pigeons would rely on familiar visual information to localize the loft. This final step of the homing process is called *local navigation* (Bingman and Able, 2002) (Fig. 4).

Despite the large body of evidence supporting olfactory navigation, some researchers attribute to magnetic cues a key role in navigation. The proposed magnetic map would be based on variations of geomagnetic intensities (Wiltschko et al., 2005; Walker, 1999).

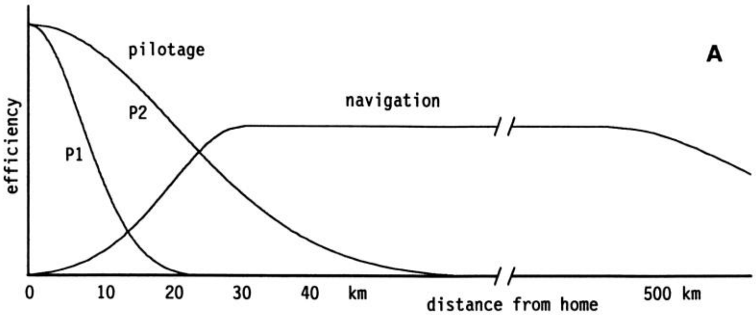

Fig. 4. Scheme showing orientational efficiency of two homing mechanisms as a function of distance from home. Range of pilotage may be different depending on the range of individual spatial experience (P1 e P2). Distances given and shapes of curves are merely indicative. In the zone of overlap, total efficiency may either correspond to the higher of the two curves or it may be even higher, depending on whether there is some cumulative effect or not. (Wallraff, 1974b).

1.4.1.2 The Magnetic map

The geomagnetic field represents a relatively reliable and omnipresent source of information. At any point on the Earth’s surface, the magnetic field can be described as a vector in three-dimensional space pointing to magnetic North. The field is derived from sources in the core and crust of the Earth. The magnetic vector provides the birds with directional information (as described before the birds perceived the direction of the magnetic field), and the spatial distribution of factors such as total intensity and inclination may provide information about position.

Because a pigeon is so small relative to the scale of variations in the Earth’s magnetic field it can be treated as a point detector capable of detecting the direction and intensity of the magnetic field vector at any point in space. Since these two variables can be used in a bi-coordinates navigation system, it has been suggested that pigeons use the direction of steepest slope in total intensity of the Earth’s magnetic field, as vector coordinates for determining position. The suggestion is that pigeons learn the direction of the magnetic gradient of the surroundings while flying around the loft and in the nearby areas during spontaneous and training flights. By comparing differences in the field between the release point and the home loft the pigeons might be able to determine their position relative to home. It has been then hypothesised that if the pigeons use such a scheme, any small artificial change in the magnetic field might be expected to produce errors in their homeward orientation (Walker, 1997; 1998; Walker et al., 2002).

The first results suggesting a role of magnetic stimuli in a position finding mechanism in homing pigeons come from experiments conducted with birds released from locations characterized by strong magnetic anomalies. At these sites an impairment in initial orientation has been observed, although not consistently. A positive correlation between the extent of magnetic variations at different sites and the degree of scattering of the tested groups has been reported by Walcott (1982). It has been suggested that the pigeons orientation errors were due to the anomaly in the local magnetic parameters, which would not be predictive of the relative site position with respect to home. Nevertheless, the results of experiments conducted in areas of magnetic anomaly produced controversial findings. Other results suggesting a role of geomagnetic field in navigation come from experiments in which the birds were exposed to an intense magnetic field of short duration (*magnetic-pulse*). It has been observed that the magnetic-pulse treatment affected the orientation of pigeons (Beason et al., 1995; 1997) and it has been proposed that the treatment involved only the map mechanism (Munro et al., 1997).

Although there are indications that many animal species rely on geomagnetic information to orient and navigate, little is known about the neural and biophysics mechanisms underlying the magnetic perception. Therefore the question about how the pigeons sense the geomagnetic information is still subject of debate. Before reporting the experimental work we have done to test the hypothesis of a role of the geomagnetic field in pigeon’s navigation, a brief overview of the hypothesized mechanisms underlying magnetoreception is given below.

Magnetoreceptors

The lack of knowledge about the morphology and the location of magnetoreceptors come partially from the nature of stimulus that they have to sense, as the magnetic stimulus easily penetrate living tissues. For these characteristics of the stimulus the magnetoreceptors do not need to be in contact with the external environment and do not need accessory structures (such as lenses for the visual sensory system). Therefore the magnetoreceptors may be small or dispersed within the body. Among the theoretical models for magnetoreception that have been proposed, the use of photoreceptor-based mechanisms involving cryptochromes in the retina and magnetite particles as magnetoreceptors are currently debated (Ritz et al., 2000; Kirschvink and Gould, 1981).

Radical Pair Model

As every chemical reaction is influenced by the magnetic field in which it takes place, it has been proposed the chemical magnetoreception hypothesis. Several ingenious mechanisms have been proposed and debated (Grissom, 1995; Lednev, 1991), but the only hypothesis that has so far gained widespread acceptance as physically plausible is one that relies on chemical reactions involving pairs of radicals (Leask, 1977). In 2000 Ritz presented a theoretical framework for a vision-based magnetoreception mechanism that connects the molecular and quantum properties of radical-pair processes with the behavioural responses of a bird. According to this model magnetoreception would occur in the retinal photoreceptors, involving chemical reactions influenced by the geomagnetic field (Ritz, 2000). Behavioural and electrophysiological studies have shown evidence that a light dependent mechanism provides directional information, which are therefore used in the compass mechanism (Semm and Demaine, 1986; Wiltschko and Wiltschko, 1995). A simple radical-pair reaction scheme, which can act as a chemical magnetoreceptor mechanism as suggested originally by Schulten and Windemuth (1986), is depicted in Fig. 5. The reaction scheme begins with an excited donor molecule D* who transfers an electron to an acceptor molecule A^-, resulting in a radical pair $D^+ + A^-$. This leaves each molecule with an unpaired electron, the spins of which are either opposite (singlet state) or parallel (triplet state). Such a spin-correlated radical pair can be generated, for example, by photoinduced electron transfer. These two states are inter-convertible and chemically different. The alignment of macromolecules in the magnetic field affects the singlet and triplet state by shifting the balance between the two states.

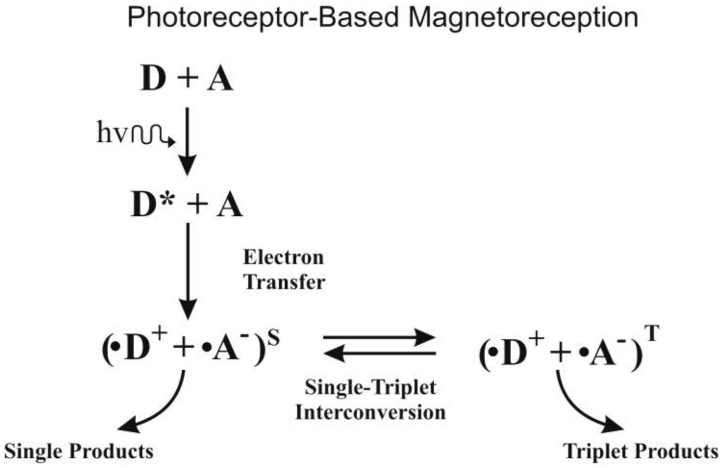

Fig. 5. Reaction scheme for a radical pair reaction with magnetic field-dependent reaction products. The radical pair is generated by an electron transfer from a donor molecule D^* to an acceptor molecule A^-. An external magnetic field affects interconversion between singlet and triplet states of the radical pair.

Therefore in summary, magnetic fields can lead to several different concentrations of singlet or triplet state. The presence of two types of products, whose mutual relationships change depending on the magnetic field, may indicate an animal's ability to evaluate the difference between the two products and then determine the intensity of the magnetic field that induced this different concentration.

However, since one of the main arguments developed in my PhD project is the involvement of the ophthalmic branch of the trigeminal nerve in the perception of changes in the magnetic field intensity, I will discuss below in more detail, the other mechanism that is thought to be involved in magnetoreception, in particular in the detection of the geomagnetic field intensity.

Magnetoreception through ferromagnetic material

The magnetite (Fe_3O_4) is a material of biogenic origin detected in honeybees, birds, salmon, sea turtles and a number of other animals that are known to be affected by the Earth's magnetic field (Kirschvink, 1985). In the birds, the magnetite particles were found in the ethmoid region of the upper beak area of the head (Williams and Wild, 2001; Fleissner et al., 2003; Beason and Nichols, 1984; Walcott et al., 1979). Most magnetite isolated from animals has the form of single-domain magnetite crystals (SD), or superparamagnetic (SP) crystals (Fig. 6). On the basis of the shape and size of particles of magnetite, different models explaining the magnetoreception have been proposed. The transduction of magnetic information through the single-domain magnetite crystals to the nervous system, may occur through a mechanical stimulus produced by the particles aligned with the magnetic field at the receptor level (Kirschvink et al., 2001; Presti and Pettigrew, 1980; Kirschvink and Gould, 1981). The superparamagnetic particles, however, have no permanent magnetic moment and do not change their position based on changes of the magnetic stimulus. These particles, developing a magnetic moment when subjected to magnetic stimulation, can generate a quite strong field likely to attract or repel the neighbouring crystals (Winklhofer, 2001; Davila et al., 2005). This interaction might deform the matrix structure in which the crystals are included, providing information about the direction and/or the intensity of the magnetic field to the nervous system.

In birds, crystals of a trivalent iron compound thought, to be magnetite, have been detected in an area of the upper beak (Fleissner et al., 2003; Beason and Brennan, 1986). In pigeons, ultrastructural analyses of this anatomical region have revealed clusters of these crystals inside nerve terminals and arranged along the cell membrane (Fleissner et al., 2003). However, in contrast to the single-domain magnetite detected in fish, the magnetite crystals in the beak of the pigeon are superparamagnetic (Winklhofer, 2001; Hanzlik and et al, 2000). An interesting similarity between fish and birds is that, in both cases, the anatomical site that contains the magnetite seems to be innervated by the ophthalmic branch of the trigeminal nerve (V branch brain) (Fleissner et al., 2003; 2007; Walker, 1997) (Fig. 7-8). It has been suggested the involvement of this nerve in the magnetic perception.

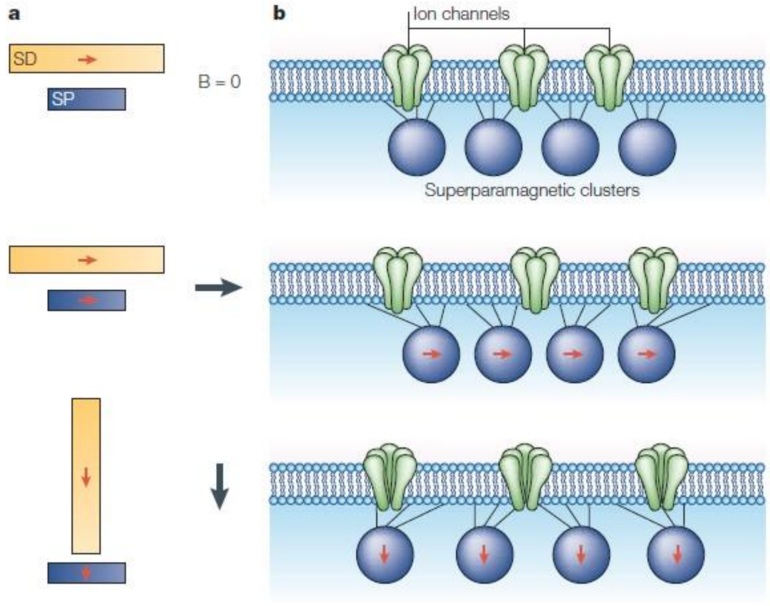

Fig. 6. The different magnetic properties of single-domain and superparamagnetic crystals. a | Single-domain (SD) and superparamagnetic (SP) magnetite crystals have different magnetic properties. Single-domain crystals have permanent magnetic moments (indicated by red arrows) even in the absence of an external magnetic field (B = 0). If an external field is present (black arrow) and the crystals are free to rotate, they will align with the external field. By contrast, superparamagnetic crystals have no magnetic moment in the absence of an external field. If an external field is present, however, the crystals develop a magnetic moment that tracks it, even though the crystal itself does not rotate. b | A hypothetical transduction mechanism based on interacting clusters of superparamagnetic crystals located in the membranes of neurons. Depending on the orientation of the external field, the clusters will either attract or repel each other, deforming the membrane and possibly opening or closing ion channels. For example, when the external field is parallel to the cell membrane, the fields in each crystal (red arrows) align in such a way that adjacent clusters attract each other like a row of bar magnets aligned end to end (middle panel). The membrane might, therefore, be slightly compressed. By contrast, a 90-degree changes in the orientation of the external field (bottom panel) results in different interactions between clusters, because adjacent clusters now behave like a row of bar magnets aligned side by side. The resulting interactions might stretch the membrane and open ion channels. (Johnsen and Lohmann, 2005).

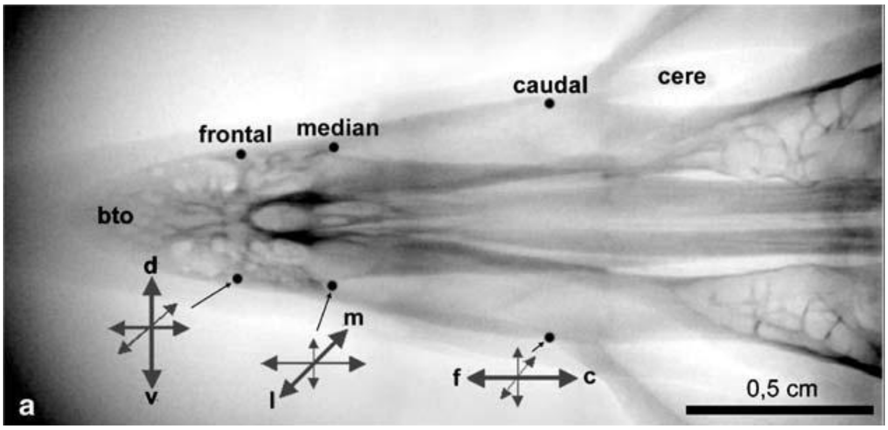

Fig. 7. Spatial distribution and quantity of iron minerals within the putative magnetoreceptor system. A X-ray image of the upper beak showing its six iron-containing areas and the prevailing orientation of their dendritic fields (arrows: c caudal, d dorsal, f frontal, l lateral, m median, r rostral, v ventral) (Fleissner et al., 2007).

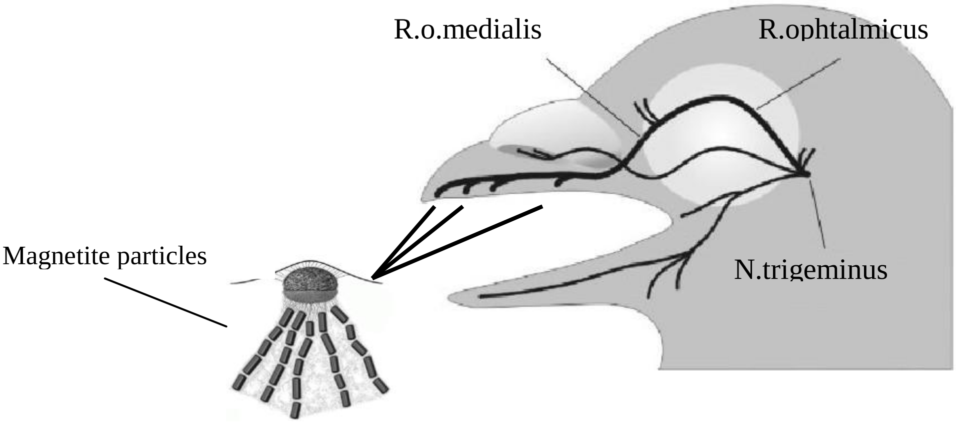

Fig. 8. Schematic representation of the magnetite particle located in the upper beak of pigeons and supposedly innervated by the ophthalmic branch of the trigeminal nerve (V branch brain).

Electrophysiological recordings in migratory birds indicate that specific neurons in the trigeminal ganglion, to which the ophthalmic branch of the trigeminal nerve projects, respond to changes in vertical field intensity as small as about 0.5% of the Earth’s field. These cells have been proposed to detect changes in magnetic field intensity useful for a navigational map mechanism (Semm and Beason, 1990).

The use of magnetic field intensity for a position finding mechanism was suggested on the basis of results produced by pulse magnetization experiments. It has been observed that a strong magnetic field of brief duration can be used to alter the direction of magnetization in single-domain magnetite particles (Kalmijn and Blakemore, 1978). It has been reported that magnetic pulse treatments, that are supposed to alter magnetite-based magnetoreceptors, affect birds’ orientation. It has also been observed that the anaesthesia of the ophthalmic branch of the trigeminal nerve in migratory birds suppresses the deviation in orientation due to the pulse magnetization treatment (Beason and Semm, 1996; Wiltschko et al., 1994b).

Other studies conducted by Mora and collaborators (2004), showed the involvement of the ophthalmic branch of the trigeminal nerve in a discriminating magnetic task in pigeons. Four pigeons had to choose which platform to go up depending on the absence or presence of a strong magnetic anomaly to obtain a food reward. Once undergoing to a nasal anaesthesia, the pigeons were no longer able to discriminate between the absence and the presence of the magnetic anomaly. Subsequently, the authors subjected two pigeons to a bilateral section of the trigeminal nerve and two pigeons to a bilateral section of the olfactory nerves. The pigeons operated to the trigeminal nerve, were no longer able to discriminate the presence from the absence of the magnetic anomaly, while the anosmic pigeons were still able to perform a magnetic discrimination. This experiment supports the role of the ophthalmic branch of the trigeminal nerve in the magnetoreception.

Gagliardo and colleagues (2006) directly compared the roles of the olfactory nerve and of the ophthalmic branch of trigeminal nerve in homing. From two sites located in opposite directions with respect to home, they released three different groups of inexperienced homing pigeons subjected to: (1) a sham operation, (2) bilateral section of the olfactory nerve, or (3) bilateral section of the ophthalmic branch of the trigeminal nerve. The experiment showed a dramatic impairment in the homing performance only for the pigeons with the olfactory nerve sectioned. In contrast, neither the sham operation nor resection of trigeminal nerve affected the capability of pigeons to return to their home loft. On the basis of these results Gagliardo and collaborators concluded that a trigeminally mediated magnetic sense, thought to be transduced using magnetite in the upper beak (Fleissner et al., 2003; Mora et al., 2004; Williams and Wild, 2001), is neither necessary nor sufficient for untrained pigeons to find their way home from unfamiliar sites. The same results were obtained by extensively training adult pigeons, and then surgically depriving them of either olfactory or trigeminally mediated magnetic information, prior to testing their navigational abilities (Gagliardo et al., 2009b). The birds deprived of trigeminally mediated magnetic information displayed similar navigational abilities as intact control pigeons, whereas the olfactory-deprived pigeons were dramatically impaired in homing. Once again, these authors showed that even in trained adult pigeons, olfactory cues are needed for homing from unfamiliar locations and that the lack of magnetic information does not affect navigational abilities of experienced adult homing pigeons. The function of the putative magnetoreceptor in ecologically relevant behaviours thus remains mysterious.

1.4.2 Navigation over familiar area

During training flights and exploratory free flights around the loft, pigeons can learn a map of the environment (Gagliardo et al., 2007a) which consists of a mental representation of the spatial distribution of familiar landmarks (Bingman and Mench, 1990). Theoretically this map can be based on the spatial distribution of various kinds of sensory cues (visual, magnetic, acoustic and olfactory). Although the visual information could be intuitively considered important in the orientation from familiar locations, the role of “visual familiar landmarks” has been subject of debate. Some authors claimed that visual feature of the landscape do not constitute orienting stimuli for homing (Schmidt-Koenig, 1991; 1979; Wiltschko and Wiltschko, 1998; Wiltschko, 1991; Keeton, 1974) for the following reasons: a) pigeons are able to orient also if released from unfamiliar locations; b) pigeons when subjected to a clock-shift treatment showed a deviation from home consistent with the phase shift if released from both unfamiliar and familiar release sites (Keeton, 1974; Wiltschko and Wiltschko, 1998); c) pigeons equipped with opaque lenses and released from familiar locations were homeward oriented (Schmidt-Koenig and Schlichte, 1972; Benvenuti and Fiaschi, 1983). In particular as regarding the deflection in initial orientation of clock-shifted birds released at familiar sites R. and W. Wiltschko stated “This means that there are no indications for a general change in navigational strategy when pigeons become familiar with a release site: their headings are not controlled by landmarks and landscape features, but continue to be determined as a compass course and located with a compass” (Wiltschko et al., 2005).

Actually clear evidences of a critical role of visual landmarks in pigeon navigation have been accumulated.

Anosmic homing pigeons, unable to navigate from unfamiliar locations, are unimpaired when released at familiar sites (Papi et al., 1973; Papi, 1982) and display good homing performances even when released at unfamiliar sites located within a familiar area (Wallraff and Neumann, 1989; Wallraff et al., 1989). Braithwhite and Guilford provided the first direct evidence that the view of the familiar release site affects pigeon performances. By using clear or opaque sided release boxes, the authors experimentally showed that pigeons allowed to observe their surroundings before release from a familiar site, could on average home faster than pigeons that were denied the same visual experience. Their results suggested an important role for the use of vision, possibly in the recognition of landmarks during the homing process (Braithwaite and Guilford, 1991). Experiments testing the orientation of pigeons in a circular arena before their take off (Mazzotto et al., 1999; Gagliardo et al., 2001b) showed that unmanipulated and anosmic pigeons were homeward oriented if they were allowed to view the release site landscape features (Gagliardo et al., 2001b). If the view of the surroundings was prevented, the control smelling pigeons still displayed a homeward orientation, while the anosmic pigeons were disoriented. These results indicate that in familiar areas pigeons can use both visual and olfactory information and that the visual cues play a major role for site recognition when the olfactory information are not available (Wallraff and Neumann, 1989; Wallraff et al., 1993; 1999; Gagliardo et al., 2001b; 2005a).

The role of familiar visual landmarks in shaping the flight path beyond the release site area has been demonstrated by experiments with GPS data loggers. Biro and colleagues (2004) intensively trained birds from two distinct release sites. Once birds had completed all releases from the training site, they were released once from four novel (‘‘offroute’’) release sites. The locations of these release sites were chosen individually for each bird such that they lay ~1–1,5 Km (perpendicular distance) from a corridor defined by the birds’ final three tracks recorded during the training phase. The results showed that after extensive experience, pigeons assume stereotyped routes home, even when released from novel sites off-route. These routes were surprisingly inefficient and were neither the most direct paths home nor similar across subjects. Thus, the recapitulated route clearly attracted birds from a distance and from novel directions and then controlled subsequent flight behaviour. The authors proposed that, after extensive experience with a particular location, pigeons build a representation of the homeward route in the form of a ‘‘route map,’’ i.e., a series of memorized visual landmarks or ‘‘waypoints”. This precise route loyalty demonstrates, again, a clear reliance of the pigeons on familiar landmarks (Biro et al., 2004).

The visual information of the familiar area can be used by the birds within two different navigational strategies, the existence of which can explain inconsistencies in results of clock-shift experiments conducted with pigeons released from familiar locations. The two strategies are described below and their relative contribution to the pigeons behavioural output can be highlighted by clock-shift experiments in which the compass information is set in conflict with the topographical information.

i) The “*site-specific compass orientation*” strategy. Pigeons adopting this strategy recall the familiar site features in association with the compass direction leading them home (Wallraff, 1974; Gagliardo et al., 1999; Holland, 2003). In this case, the birds use the local familiar landmarks only to recall a compass direction. In this case the navigational process follows the typical “map and compass” steps.

ii) The "*piloting*" strategy (Baker, 1984; Papi, 1992a). Pigeons adopting this strategy rely on the spatial relationships among the familiar landmarks (Holland, 2003). These relationships are memorized to constitute a topographical map that, without the aid of compass, allows the animal to find its way home. This topographic map could be interpreted as cognitive map (Bingman and Able, 2002; O'Keefe and Nadel, 1978).

As concerning the role of familiar visual landmarks in correcting the navigational error following clock shift treatments, several studies have shown a large degree of variability in the observed deviations in the pigeons' initial orientation. In some experiments, the pigeons showed a deviation consistent with the expected (Graue, 1963; Luschi and Dall'Antonia, 1993; Keeton, 1969), while in other experiments the observed deviation was lower or null with respect to the expected values (Bingman and Ioalè, 1989; Wallraff, 1994; Gagliardo et al., 1999; 2002; 2005a; Wiltschko and Wiltschko, 2001a). It is worth noting that the greater degree of deviation observed with respect to the expected deviation, has been observed in pigeons released under anosmia and therefore forced to rely on familiar visual landmarks for navigation. During experiments aiming at investigating which factors are involved in the reduction of the deviation due to the clock-shift treatment, Gagliardo and colleagues (2005a) performed a series of releases with intact and anosmic pigeons from familiar sites in un-shifted and clock-shifted conditions. It has been shown that the two groups differed in the extent of their deviations from the home direction. The deviations observed for the anosmic pigeons were significantly lower than those observed for the untreated control birds. Gagliardo and colleagues interpreted the reduction of the deviations observed in theirs pigeons as mainly due to the use of topographical information within a piloting strategy. Also the smelling birds released from familiar sites displayed a reduced deflection in their initial orientation, although to a lesser extent if compared to the anosmic birds. This was due to the familiarity with the release sites, as suggested by the greater deflection of birds released for the first time at the same sites. In conclusion, at familiar sites the anosmic birds seem to rely preferentially on a piloting strategy, while the smelling pigeons seem to prefer a "site specific compass orientation" strategy, probably also using information provided by the olfactory map mechanism. From the data in the literature emerged that the pigeons tend to preferentially rely to the site specific-compass orientation strategy (Gagliardo et al., 1996; Chappell and Guilford, 1995). However it has been shown that pigeons can make a compromise between the two strategies and that there are factors making them shifting to the piloting strategy. In experiments conducted with route recorders on clock-shifted pigeons released from familiar sites (Bonadonna et al., 2000; Holland et al., 2000) it was shown that some pigeons flew directly towards home, while other birds made wide detours due to the wrong information provided by the sun compass. This suggests that there is an individual variability in the preferred strategy. Furthermore, the preference for one or the other strategy may also depend on the characteristics of the release site and the over-flown area (Bonadonna et al., 2000; Gagliardo et al., 2005a). Biro and colleagues (2007) examined how homing pigeons' reliance on sun-compass information changes with the level of familiarity with the area. Two groups of homing pigeons with different level of familiarity, one subjected to intensive training and one with a limited experience have been tested after clock shift. It has been observed that birds with a higher level of experience tend to follow the same route memorised during the training flights, while the less experienced birds display a lower level of route fidelity. Therefore, given sufficient experience, homing pigeons develop individually distinct, stereotyped routes home, which they accurately recapitulate on each subsequent journey. These findings indicate that memorized visual landmarks can directly provide onward guidance along the entire route home and not just site recognition cues at release. However, when birds are less familiar with the environment, the site specific compass orientation becomes dominant, suggesting that previous experience establish which mechanism is used.

An important role in birds spatial behaviour is played by the hippocampal formation (HF), similarly to what happens in mammals (O'Keefe and Nadel, 1978; Bingman et al., 2006).

The first study on the effects of hippocampal lesions, in homing pigeons performance, was performed by Bingman and colleagues in 1984. This and subsequent studies showed that lesioned pigeons released from unfamiliar locations exhibited a good initial orientation but slower homing performances, likely due to difficulties in the localization of the loft within the home area (Bingman et al., 1984; 1988; Bingman and Mench, 1990; Strasser et al., 1998). The fact that the lesioned pigeons, exhibit an initial orientation towards home when released even from distant unfamiliar places, indicates that the hippocampus is not involved in a position finding system, once the navigational map is learned. The lower efficiency of the hippocampal ablated pigeons in localising the loft suggests an involvement of the hippocampal formation in landmark based navigation, which is requested in the last phase of the homing process (Gagliardo et al., 2007c; Bingman and Mench, 1990; Bingman et al., 1990). It was observed that hippocampal lesioned pigeons are unable to reorient on the basis of the familiar landmarks array (piloting strategy) (Gagliardo et al., 1999). Pigeons with hippocampal lesions were trained in groups from two places located in opposite directions from home. Before the experimental tests the pigeons were made anosmic in order to prevent them from using the olfactory navigational map. The pigeons were also subjected to a phase shift treatment. Therefore, the only information available for re-orientation was given by the familiar topography. While the intact anosmic pigeons displayed a tendency to orient homeward, therefore relying on the spatial relationships among landmarks memorized during the previous homing flights, the lesioned pigeons displayed a deviation from the home direction as expected on the basis of the use of a site specific compass orientation strategy. These results were confirmed by a GPS study (Gagliardo et al., 2009a). During this experiments it has been highlighted the important role of the sea as a landscape boundary in re-orientation. The fact that HF-lesioned pigeons (no control) spent a considerable amount of time flying over the sea on their way from a release site located close to the coast, suggested that rather than point source landmarks, coarse landscape features and the ease with which different landscape features may be segmented from other landscape features, play an important role in re-orientation. Moreover, the behaviour of the HF-lesioned pigeons suggests, as already shown in previously experiments, that they are specifically diminished in their capacity to use such landscape features for navigation.

The involvement of the hippocampal formation in a landmark based navigation during homing has been also recently highlighted in a study in which it has been investigates the expression of the immediate early gene ZENK in birds unilaterally occluded or not and released at unfamiliar site or transported to the site without being released. The results revealed that the hippocampus is activated in both flying groups, but not in those just kept at the release site. These results are consistent with hippocampal recruitment in the local navigation and memorization of topographical cues during homing (Patzke et al., 2010).

1.5 THE OLFACTORY SISTEM OF BIRDS

1.5.1 Neural basis of olfactory navigation

For a long time, the olfactory sense of smell in birds' biology was considered unimportant or not even functional. The small size of the olfactory bulbs (Obs) in relation to the rest of the brain in many species of birds was interpreted as a hint for a rather underdeveloped sensory system (Roper, 1999). However, comparative anatomical studies revealed a high degree of similarity between the avian olfactory system and those of amphibians, reptiles and mammals both on the macroscopic and microscopic level (Roper, 1999). Recently it was also demonstrated that the majority of avian olfactory receptor genes are potentially functional (Steiger et al., 2008) and that they are not, as previously believed, non-functional pseudogenes.

The olfactory receptor cells located in the olfactory chamber constitute the olfactory mucosa. The olfactory epithelium includes several distinct cell types: the olfactory receptor neuron, basal cells and supporting cells. The olfactory receptor cells are bipolar neurons provided with cilia which protrude from the olfactory receptor cell's dendrite into the mucus covering the surface of the olfactory epithelium. These cells differentiate continuously from the basal cells located above the olfactory mucosa. When the primary olfactory neurons degenerate new cells differentiate from the basal cells. By consequence a turnover of olfactory neurons is guaranteed by the differentiation and the replication of basal cells. The olfactory receptor cells are connected with a pair of olfactory nerves with the olfactory bulbs, which are located at the rostral end of the brain (Rieke and Wenzel, 1978). The OBs project at various brain areas as follows. They project bilaterally to the prepiriform cortex (CPP), nucleus taeniae of the amygdala (TnA), CPi, dorsolateral corticoid area (CDL), and ipsilaterally to the medial striatum (SM). The piriform cortex that is supposed to be involved in processing olfactory information is connected to other main telencephalic areas: the CPP, the OBs, the dorsal acropallium (AD), the hyperpallium densocellulare (HD), the hyperpallium laterale (HL), and the frontolateral nidopallium (NFL) (Fig. 9) (Patzke et al., 2011; Reiner and Karten, 1985; Bingman et al., 1984).

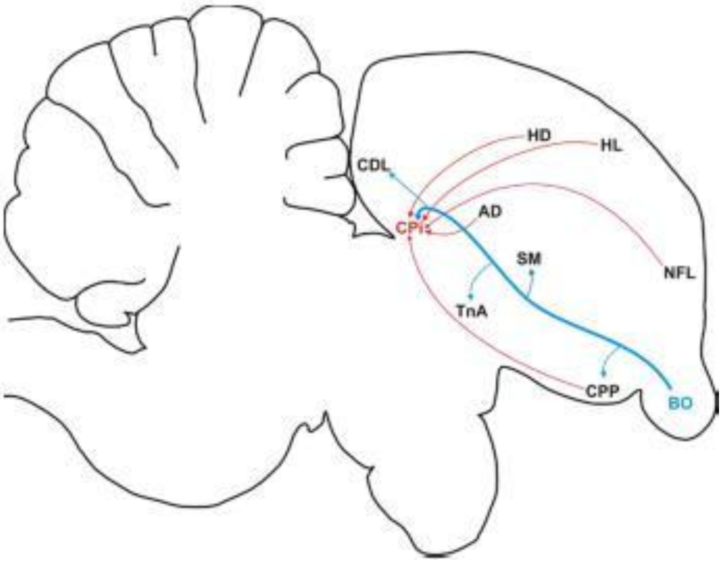

Fig. 9 Schematic representation of the main telencephalic olfactory projections. The OB project bilaterally to the CPP, TnA, CPi, CDL, and ipsilaterally to the SM. In addition to that, CPi receives bilateral input from CPP and ipsilateral input from AD, HD, HL, and NFL. From (Patzke et al., 2011).

As previously mentioned the involvement of the olfactory sense in navigation was discovered by Papi and colleagues in the 1972, by observing a dramatic impairment of pigeons' navigational abilities as consequence of anosmia (Papi et al., 1972). Subsequent studies have shown that besides the section of the olfactory nerves, and the disruption of the olfactory mucosa, also lesions to brain areas processing olfactory information, as the piriform cortex (CPi) disrupt pigeons homing abilities. However, bilateral ablations of the CPi impair pigeons' homing ability only from unfamiliar sites, but not from familiar site, suggesting a specific role of this brain region in true navigation. Accordingly, lesions of the Cpi in young birds prevent the development of the olfactory map even when the pigeons are allowed to perform spontaneous flight around the loft (Gagliardo et al., 1997).

Besides the role of olfactory cue in navigation, in the last thirty years many behavioural studies have provided a large body of evidence about the involvement of the sense of smell in birds' biology. Scents play a role in food location in kiwis and vultures (Balthazart and Taziaux, 2009) but also in recognition of familiar places like the nest in domestic fowl chicks (Porter et al., 1999), petrels (Hagelin and Jones, 2007), in courtship in ducks (Balthazart and Taziaux, 2009), in finding burrow and partner-odour recognition in Procellariiform seabirds (Bonadonna and Nevitt, 2004), and in natal nest recognition (Caspers and Krazuse, 2010). Moreover, chemical signals have been shown to play a major role in sex recognition (Bonadonna, 2009) and in specific partner's recognition (Bonadonna et al., 2007).

1.5.2 Functional lateralization of the olfactory system

The functional and structural lateralization of the left and right hemisphere is not only a feature of the human brain but is common in the entire animal kingdom from the fruit fly to mammals (Vallortigara et al., 1999; Vallortigara and Rogers, 2005). Having a lateralized brain is considered to enhance the efficiency and neuronal capacity of the brain, by reducing the interhemispheric conflict. In birds, one of the most extensively studied lateralized systems, is the visual system, which reveals functional and anatomical lateralization (Manns and Güntürkün, 2009). However, while the visual lateralization in birds is a well studied phenomenon, the olfactory lateralization is poorly understood. In fact only recently the question of a possible functional asymmetry in the olfactory system was addressed in birds.

The first evidence of a functional lateralization in the olfactory system of birds was provided by Giorgio Vallortigara and Richard Andrew in chicks exposed to olfactory stimuli during imprinting. Their study showed that chicks performed better in a discrimination task between an olfactory familiar and unfamiliar rearing object, when they could smell with the right nostril (Vallortigara and Andrew, 1994). In 1996, Benvenuti and Gagliardo provided a report addressing a possible olfactory lateralization in pigeons with previous homing experience. In this experiment birds with extensive homing experience subjected to unilateral anosmia (zinc-sulphate treatment) and unilateral nostril occlusion, were released from unfamiliar locations. The experiment aimed at testing possible non specific effects of the zinc-sulphate treatment. Therefore for the anosmic birds the plugged nostril was contralateral to the zinc-sulphate treatment, while for the controls the plugged nostril was ipsilateral to the zinc-sulphate treated side. Consistently with previous results, bilateral anosmia disrupted the navigational performances of the birds. As regarding lateralization, no difference in terms of initial orientation and homing rates between the left-side and the right-side treated birds was highlighted. Therefore the authors concluded that there was no evidence for olfactory lateralization in homing pigeons (Benvenuti and Gagliardo, 1996).

In the following decade, Gagliardo and collaborators conducted other experiments aimed at investigating olfactory lateralization in pigeon navigation tasks. Since it was known that an intact Cpi is required to navigate from an unfamiliar locations (Papi and Casini, 1990), Gagliardo in 2005 examined the effects of unilateral lesions of the CPi in adult inexperienced birds (Gagliardo et al., 2005). Pigeons with an intact left Cpi showed an unimpaired initial orientation compared to the control birds, while pigeons with an intact right Cpi were randomly scattered. This suggested that there is a functional dominance of the left Cpi in processing olfactory cues for the determination of the direction of displacement. However, as the ablation induces comparable homing performances in both treated groups, it was hypothesized that the right Cpi might provide an important contribution in the navigation process *en route*. However this hypothesis still needs to be tested.

A subsequent study was carried out with naïve pigeons made anosmic by means of a unilateral nostril occlusion (Gagliardo et al., 2007c). Adult inexperienced birds were divided in three experimental groups: left nostril plugged pigeons (LNP), right nostril plugged pigeons (RNP) and unmanipulated pigeons (C). The unilaterally plugged birds which had returned from the first release were tested from a second and more distant site after switching the side of the plug, together with the unmanipulated control group. The birds with the left nostril occluded showed unimpaired initial orientation performances. By contrast, the pigeons smelling with the left nostril (right nostril occluded) were impaired in their initial orientation. These two studies highlighted a functional asymmetry in favour of the right nostril/left CPi (Gagliardo et al., 2005b; 2007b), suggesting a possible involvement of the contralateral OB-

CPi projections. It is worth-noting that different physiological studies have shown that the major projections from the OB to the CPi are ipsilateral rather than contralateral (Rieke and Wenzel, 1975; 1978; Reiner and Karten, 1985; Bingman et al., 1994; Patzke et al., 2011). Moreover, Patzke and colleagues observed that both contralateral projections from the OB to higher brain areas are symmetrically organised and therefore the functional asymmetry observed at behavioural level cannot explained at a neuroanatomical level (Patzke et al., 2011). Another investigation (Patzke et al., 2010) of the same issue has involved the expression of the immediate early gene ZENK in birds released at unfamiliar site or transported to the site without being released. To evaluate the differential contribution of the left and ⁄ or right olfactory input, the nostrils of the pigeons were either occluded unilaterally or not. The results showed for the unplugged birds released from unfamiliar sites a comparable activation between the two hemispheres of the OB and the CPi. However, both Cpis seem to contribute differently to the navigation process. Only occlusion of the right OB resulted in a decreased ZENK cell expression in the Cpi, whereas occlusion of the left nostril had no effect. This study reveals for the first time a neuronal activation patterns in the olfactory system during homing with a stronger connectivity between the OB and the CPi in the left rather than in the right hemisphere.

1.6 INVESTIGATION OF HOMING PIGEONS: CLASSICAL APPROACH AND NEW TECHNOLOGIES.

In the experiments discussed in this research project, have been applied two different methods of investigation: classical investigation methodologies and innovative methods.

1.6.1 Classical methods

When we talk about classical methods of investigation, we refer to experimental protocols that provide direct observation of the orientation of the pigeon at the release site and the timing of return to the aviary.

A traditional release experiment on homing pigeon is conducted as follows. A number of pigeons, individually labelled by numbered rings and selected according to age, homing experience, group membership, etc., are caught in their home loft and confined in baskets or crates. The pigeons are transported to the release site, where they are released individually. Each bird is tossed into the air and observed with binoculars until it vanishes from sight. Then direction and time of vanishing is recorded. At this time a pigeon is approximately 1.6 - 2.2 Km distant from the starting point (Wallraff, 2005a). During the experiment, another observer records the individual bird's time of arrival at the loft, so it is possible to calculate the homing performances, i.e. homing duration and homing rate.

However, although the observation of vanishing bearings represent an important method, which becomes highly reliable and meaningful in those cases in which the initial orientation data can be interpreted considering also the homing performance of the released birds, the development of satellite technology has made possible many advances in the study of the birds' behaviour.

1.6.2 GPS data-logger

The GPS loggers are miniature high-precision instruments equipped with an antenna, receiving signals from 25 satellites placed into orbit by the U.S. Department of Defence. The location data (longitude, latitude, altitude, time and date) are stored in a logger with 8 Mb of memory. GPS was originally intended for military applications, but in the 1980s, the government made the system available for civilian use. The GPS satellites circle the Earth twice a day in a very precise orbit and transmit signal information to earth. The GPS receivers keep this information and use a triangulation's method to calculate the user's exact location and are supposed to work in any weather conditions, anywhere in the world, 24 hours a day. Essentially, the GPS receiver compares the time at which a signal was transmitted by a satellite with the time at which it was received. The time difference tells the GPS receiver how far away the satellite is. With distance measurements from a few more satellites, the receiver can determine the user's position and display it on the unit's electronic map. A GPS receiver must be locked on to the signal of at least three satellites to calculate a 2D position (latitude and longitude); with four or more satellites in view, the receiver can determine the user's 3D position (latitude, longitude and altitude). Once the user's position has been determined, the GPS unit can calculate other information, such as speed, bearing, track, trip distance, distance to destination and more. The accuracy of the recorded position is about 5m, and depends on the number of satellites received from the GPS. The recorded data may be acquired by the computer in different formats (Binary, Text, NMEA) using dedicated software. All the GPS tracks were visualized with Google Earth (Google Inc, US).

Application on homing pigeons

The GPS data logger used for studies involving animals of small size, as in this case, are lightweight tracking system with a weight of about 20g (55x33x11mm), including battery and casing. The homing pigeons fly carrying these weights without any stress as they are accustomed to bring in their crop the food picked up as reserves. Nevertheless, to accustom the pigeons to fly with a load on its back it is necessary to equip them with PVC dummies having the same size and weight as the loggers (Fig. 10). The dummy is attached to the pigeons' back by means of a Velcro strip glued on the feathers, which must be previously trim. Before the releases, the dummy is replaced with a miniature GPS data logger in order to record the bird's flight path (Fig.11).

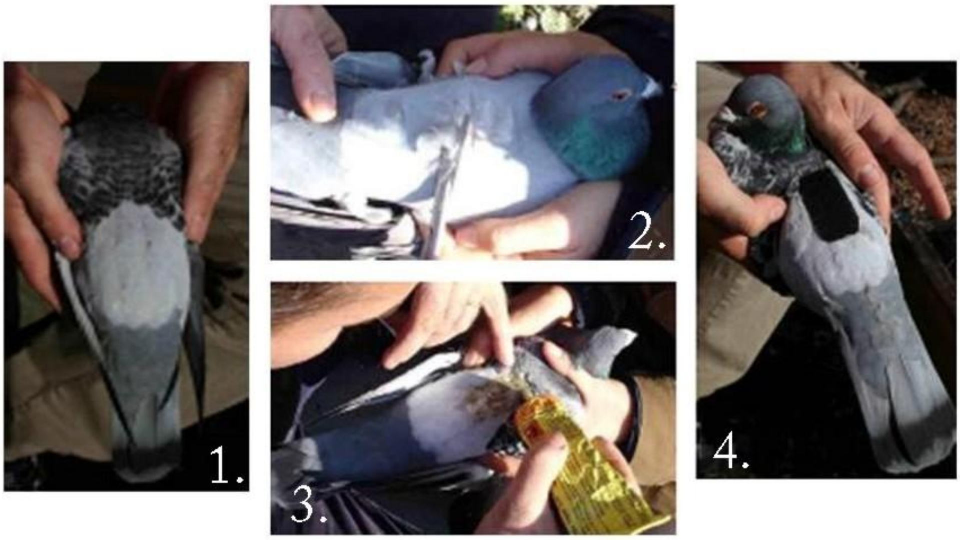

Fig.10. Application of PVC dummies on pigeon's back. 1.Preparation of the pigeon. 2. Shortening of the feathers. 3.Application of the glue and Velcro strip. 4.Positioning of the dummies.

The GPS data logger allows to record the precise homing track of the birds and therefore can provide information on the behaviour of the birds well beyond the release site.

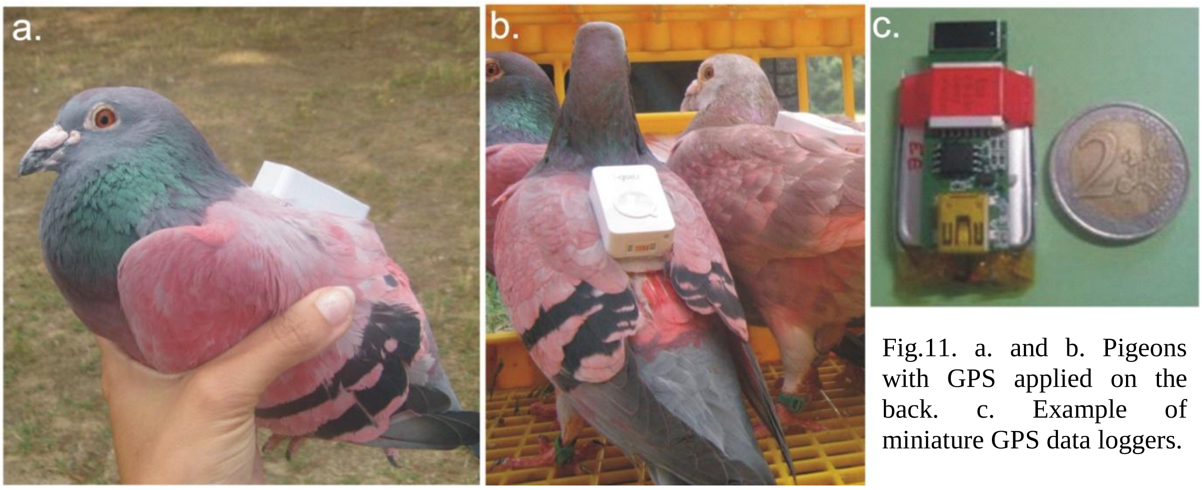

Fig.11. a. and b. Pigeons with GPS applied on the back. c. Example of miniature GPS data loggers.

1.7 PURPOSE OF THE PRESENT STUDY

The present GPS studies aim at investigating some aspects of pigeon navigation that cannot be addressed by performing traditional experiments.

First study: Olfactory lateralization in pigeon navigation.

Due to the relevance of the odour cues in pigeons' navigation, homing pigeon represent a unique animal model to study brain asymmetries in the olfactory system in natural tasks. We have analysed the tracks of birds released with the left or the right nostril occluded and we have highlighted an important functional asymmetry in favour of the right nostril. In fact the birds processing the environmental olfactory information with the left nostril only, displayed a higher level of tortuosity in their flight path and stopped more frequently than both the unmanipulated controls and the birds using the right nostril.

Second study: Role of environmental odours on the navigational map.

It has been shown that the integrity of the olfactory system is necessary for pigeon's homing. However the role of environmental odours in the position finding mechanism of homing pigeons is subject of a lively scientific debate. The olfactory navigation hypothesis proposes that environmental odours are a specific component of the navigational map, while the olfactory activation hypothesis explain the behavioural impairment of the anosmic birds, arguing that the olfactory sensation is needed to activate a navigational mechanism based on non olfactory cues. We have analysed the GPS tracks of three groups of pigeons subjected to different olfactory conditions during transportation and at the release site and subjected to nasal anaesthesia prior release: controls birds exposed to environmental odours, birds transported in pure air and pigeons transported in pure air but stimulated with artificial odour of plant origin, before the release. The analysis of the tracks revealed that the birds exposed to the artificial odours displayed significantly poorer navigational performances than controls, suggesting a specific role of environmental olfactory information in pigeon navigation.

Third study: Role of geomagnetic information in a position finding mechanism in homing pigeons.

Anatomical studies and conditioning experiments provided evidence that pigeons detect geomagnetic field intensity through the ophthalmic branch of the trigeminal nerve. Despite the fact that magnetic treatments and trigeminal nerve section do not disrupt the abilities of pigeons to home back to the colony, it has been proposed that pigeons tend to fly parallel or perpendicular to the steepest magnetic slope. We have analysed tracks of both intact and trigeminal sectioned pigeons in order to test if the pattern of the local magnetic gradient affect the birds' flight paths. The analysis did not reveal a consistent effect of the local geomagnetic field in the birds' homing trajectories.

Fourth study: Role of the topography in the landmark based navigation over familiar areas.

It is known that pigeons are able to memorise landscape features of the over-flown areas. These features can be associated to a specific compass direction leading the bird home (site specific compass orientation). Alternatively the bird can learn the spatial relationships among the single landmarks so to build a familiar landmark based map used in a piloting strategy. The two different strategies can be put in conflict by shifting the birds' internal clock, in order to asses which of the two strategies is preferentially adopted by the subject. This protocol has been used to assess which factors are determining the strategy preferentially used by an individual pigeon and the kind of landscape feature which are likely to be memorised as landmarks during piloting. The analysis of the tracks suggested that the characteristic features of the release site affect the level of reorientation after clock shift, and, in particular, the vicinity of the sea seems to determine a preference for the piloting strategy.

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2. EXPERIMENTS

2.1 OLFACTORY LATERALIZATION IN HOMING PIGEONS: A GPS STUDY ON BIRDS RELEASED WITH UNILATERAL OLFACTORY INPUTS

ABSTRACT

A large body of evidence has shown that pigeons rely on an olfactory-based navigational map when homing from unfamiliar locations. Previous studies on pigeons released with one nostril occluded highlighted an asymmetry in favors of the right nostril, particularly concerning the initial orientation performance of naïve birds. Nevertheless, all pigeons experiencing only unilateral olfactory input showed impaired homing, regardless of the side of the occluded nostril. So far this phenomenon has been documented only by observing the birds' vanishing bearings. In the present work we recorded the flight tracks of pigeons with previous homing experience equipped with a GPS data logger and released from an unfamiliar location with the right or the left nostril occluded. The analysis of the tracks revealed that the flight path of the birds with the right nostril occluded was more tortuous than that of unmanipulated controls. Moreover, the pigeons smelling with the left nostril interrupted their journey significantly more frequently and displayed more exploratory activity than the control birds, e.g. during flights around a stopover site. These data suggest a more important involvement of the right olfactory system in processing the olfactory information needed for the operation of the navigational map.

INTRODUCTION

Since the olfactory navigation hypothesis was first proposed by Floriano Papi (Papi et al., 1971), a large body of evidence supporting the crucial role of olfactory cues in navigation has accumulated (Wallraff, 2005). This hypothesis predicts that pigeons learn the association between the environmental odours carried by the winds with the direction from which they blow (Ioalè et al., 1990), so individuals can build up a map of the region around the home area on the basis of the distribution of olfactory cues (Wallraff and Andreae, 2000). When birds are displaced, the identification of the prevalent local odours provides the pigeons with information about the direction of displacement. Therefore, the released birds are able to orient towards home using a compass mechanism (Keeton, 1971; Schmidt-Koenig, 1961). The strongest evidence in favour of the olfactory navigation hypothesis comes from two main kinds of experimental protocols: (1) the manipulation of the olfactory system in order to prevent odour perception prior to release (Gagliardo et al., 2009; Papi, 1982; Wallraff et al.,

1989) and (2) the manipulation of olfactory information, either in terms of odour quality or wind direction at the home loft, aimed at preventing or manipulating map learning (Ioalè et al., 1978, 1990; Odetti et al., 2003; Papi et al., 1974; Papi, 1986; Wallraff, 1979). The latter, applied on birds with an intact olfactory system, provided results supporting a specific role of olfactory cues in navigation and contradicting the olfactory activation hypothesis, which attributes to the olfactory stimuli a role in priming a non-olfactory navigational mechanism (Jorge et al., 2009).

Because of the relevance of olfactory stimuli in spatial behaviour on a large scale, homing pigeons represent an excellent animal model for investigating functional asymmetry in the avian olfactory system, although this issue was taken into consideration only recently. The first evidence of functional lateralization in the avian olfactory system was provided in chicks exposed to olfactory stimuli during imprinting (Vallortigara and Andrew, 1994). This study revealed that chicks performed better in a discrimination task between olfactory familiar and unfamiliar rearing objects when the olfactory input was conveyed through the right nostril. An advantage of the right nostril was also reported in relation to the head shaking response, following the presentation of a high concentration of eugenol (Rogers et al., 1998). The first comparison of initial orientation and homing performance from unfamiliar sites in pigeons receiving unilateral olfactory inputs (Benvenuti and Gagliardo, 1996) was done on birds with previous extensive homing experience and it did not highlight any functional asymmetry. A more recent study on naïve pigeons showed a clear advantage in favour of the right nostril (Gagliardo et al., 2007), with regard to their initial orientation. The homing success of the birds with unilateral nostril occlusion was, however, lower than controls, regardless of the side of the treatment.

In order to investigate the behaviour en route of birds receiving a unilateral olfactory input, we performed a homing experiment from an unfamiliar location using birds equipped with GPS data loggers and released with one nostril occluded. So far the effect of unilateral occlusion of the nostrils on orientation has been documented by recording the vanishing bearings of naïve birds (Gagliardo et al., 2007), whereas in the present work we used pigeons with previous homing experience. In this way we increased the probability of all birds returning with their loggers, although we did not expect any difference between the groups with right and left nostril occlusion (Benvenuti and Gagliardo, 1996).

MATERIALS AND METHODS

General procedure

Thirty-one pigeons, about 12-18 months of age and hatched at the Arnino field station (latitude 43° 39’ 26’’ N; longitude 10° 18’ 14’’ E), Pisa, Italy, were used in the study. The pigeons were raised as free flyers and were kept and manipulated according to Italian law on animal welfare. Individuals had a single homing experience as they had been released once from 40 km North or South from home one year before the experiment. The experiments took place in July 2008. The pigeons were divided into three experimental groups: C unmanipulated control pigeons (n= 9), RNP pigeons released with the right nostril plugged (n= 9), LNP pigeons released with the left nostril plugged (n= 10). Twenty days prior to the experimental releases all the birds were equipped with a PVC dummy weight, similar in dimension and weight to the GPS data logger they would be carrying, in order to accustom them to flying with a load. The dummy was attached to the pigeons’ back by means of a Velcro strip glued on the feathers, which had been previously trimmed.

GPS data logger

We used miniature GPS data loggers (www.technosmart.eu) for recording the positional data of flying birds with an accuracy of about 4 m (Steiner et al., 2000; Lipp et al., 2004). For the current study, the GPS data loggers stored one position fix every 10 seconds. However, in one case (LNP pigeon #520) a device was, for a short period, unable to receive a satellite signal. During such a recording gap, a straight flight path between interrupted fix points is visualised in the track and that portion was not included in the analysis. The positional fixes stored by a GPS data logger include latitude, longitude, and time of recording. The devices also provide information about altitude, but with insufficient precision to allow a reliable analysis. The tracks for each pigeon for each recorded release were visualised with MapInfo.

Test releases

The evening before the experimental releases one nostril of the RNP and LNP pigeons was plugged. The plugs were made with a small amount of paste (Xantopren®) which turns into a solid rubbery plug after insertion into the nostril. The plugs were removed once the pigeons homed. If during the night some pigeons had lost the plug, it was replaced in the morning before the displacement to the release site. From our preliminary observations the pigeons are able to expel the plug within a few days. Therefore we assume pigeons which did not return to their home loft eventually lost their plug.

All birds were released from Cigoli (latitude 43° 40’ 34’’ N; longitude 10° 49’ 19’’ E; home direction 267°, home distance 41.6 km). Just prior to release, the dummy on the back of each pigeon was replaced with a GPS data logger. Each pigeon was released singly, with at least 30 minutes between releases. All the experimental releases took place under sunny conditions, with no or light wind.

Quantitative analyses and statistical procedures

All tracks shorter than 15 km were excluded from the analysis. For each pigeon we considered in the analysis the following aspects of the flight path: the track length, the stops and the tortuosity of the path. Moreover we analysed the virtual vanishing bearing at 2 km from the release site.

Efficiency index. To compare the length of the homing journey we considered the efficiency index, which we calculated as the ratio between the track length and the beeline distance between the release site and home. When the tracks were not complete we added the linear distance from the end of the track to the loft. The efficiency indices of the experimental groups were compared with the Kruskall-Wallis test. The Dunn’s test was used for multiple comparisons.

Behaviour at the stop sites. We considered as “stop site” a location where a bird interrupted its flight. A stop site was identified by visually inspecting the track at a high magnification and we considered that a pigeon left a stop site when it departed for more than 500 meters without coming back. In the analysis we compared the number of stops relative to the recorded track length with the Kruskall-Wallis test. The Dunn’s test was used for multiple comparisons.

As at a stop site the birds, besides sitting, also flew over the same area without progressing their journey, we assumed that a bird was performing exploratory flights when the recorded speed was greater or equal to 20 km/h. For each stop performed by each bird the length of the path flown at a speed of at least 20 km/h was calculated. When such a path exceeded 500 meters then we assumed that a bird in that particular stop had performed an exploratory behaviour and we considered that stop as an exploratory stop. It should be noticed that a movement at a speed of at least 20 km/h was always preceded and followed by movements at a lower speed (18-10 km/h) before landing and after taking off. For the analysis, we assigned a score to each bird depending on the number of the exploratory stops performed; the scores of the groups were compared with the Kruskall-Wallis test. The Dunn's test was used for multiple comparisons.

The percentage of time spent by the three groups of birds at the stop sites relative to the duration of the recorded flight was compared with the Kruskall-Wallis test and the Dunn's test was used for multiple comparisons.

Tortuosity. To perform this analysis we drew concentric circles, around the release site, having a radius increasing by one km. The analysis was performed from the ring ranging from 1 to 2 km up to the ring ranging from 29 to 30 km. We considered separately the portions of the tracks included in the ring delimited by two consecutive circles as reported in Fig. 1. For each portion we considered the direction taken by the bird moving from one point to the next and therefore we calculated the mean vector. The mean vector length is a suitable parameter for indicating a directional stability, because it tends to be small when the birds frequently change their direction and tends to be near to 1 when they maintain a stable direction. Therefore, we applied a two way repeated measure ANOVA on the mean vector lengths relative to portions of the tracks recorded at increasing distances in order to compare the tortuosity in the flight path of the three experimental groups. The Student-Neuman-Kewls method was applied for multiple comparisons. This analysis was made after having excluded the portions of the tracks recorded at the stop sites.

Virtual vanishing bearings and virtual vanishing and homing times. We recorded the direction of the birds at both 1 and 2 km from the release site, the latter corresponding to the virtual vanishing bearing. The circular distributions were tested for randomness by means of both the Rayleigh and V test and compared with the Mardia-Watson-Wheeler test (Batschelet, 1981). In addition we have compared the virtual vanishing times (the time taken by the bird to get 2 km away from the release site), and the homing times of the three experimental groups by using the Kruskall-Wallis test.

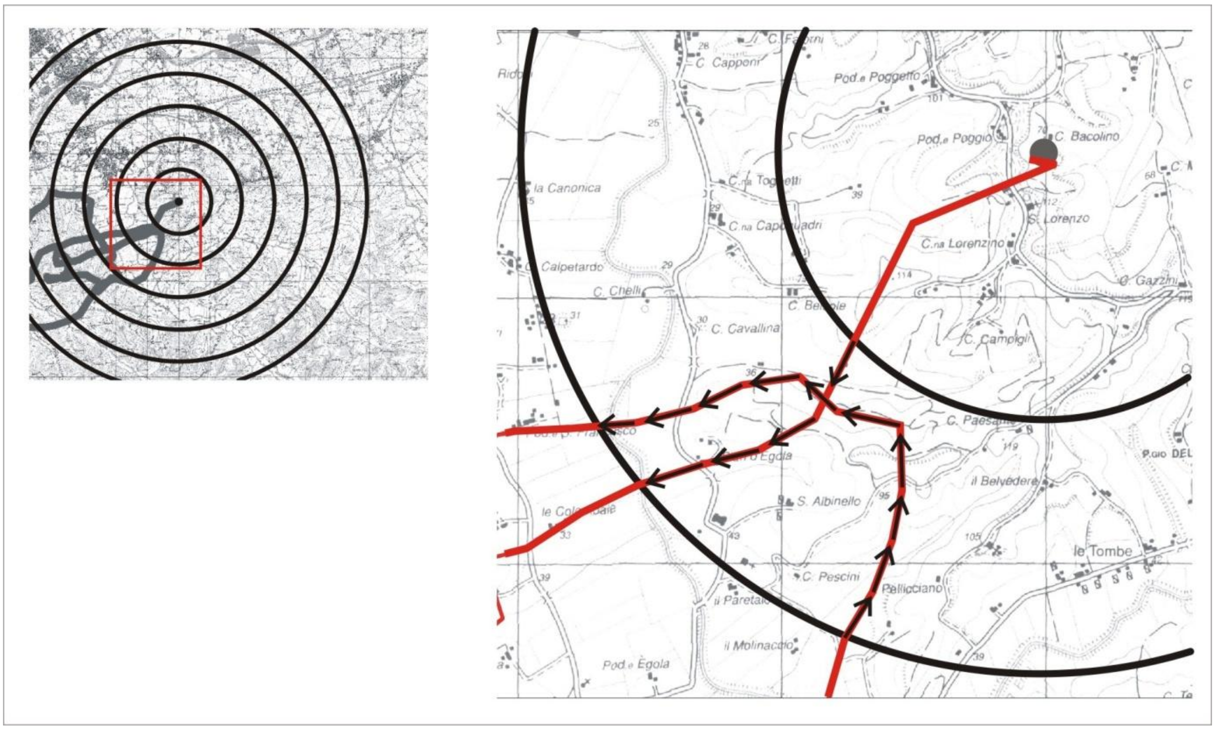

Fig.1. Example of how a track was divided into portions for the analysis of tortuosity. Each portion included the tracts of the path falling within the ring delimited between two successive circles of 1km increasing radius (left panel). The analysis was performed from the ring ranging from 1 to 2km up to the ring ranging from 29 to 30km. In the right panel, a section of the track falling within the first ring is represented. The arrows represent the directions taken by the bird flying from one fix to the next, which have been used to calculate a mean vector.

RESULTS

One C pigeon returned without GPS and one RNP and one LNP never homed. Four tracks (one C and three LNP) were shorter than 15 km and were excluded from the analysis. Therefore we obtained 7 tracks for C, 6 tracks for LNP and 8 tracks for RNP groups, respectively (see Fig. 2A-F).

Stops. The number of stops for each pigeon and the locations of the stops can be observed in Fig. 2A-F. In the same figure a magnification of a track at a stop site is reported as an example. The median number of stops/km was as follows: C 0.03; RNP 0.10; LNP 0.06. The Kruskall-Wallis test applied on the number of stops relative to the recorded track length revealed a statistically significant difference ($p<0.05$). In particular the RNP stopped significantly more often than the C-birds (Dunn’s test: RNP vs. C $p<0.05$). No difference emerged between the LNP and the other two groups.

As regards the exploratory behaviour at the stop sites, the median scores were as follows: RNP 3, LNP 1.5, and C 0. The Kruskall-Wallis revealed a significant difference between groups ($p = 0.031$). In particular the RNP pigeons were significantly different from C

(Dunn's test $p<0.05$), but not from the LNP pigeons. No difference emerged between the LNP and C groups.

According to the Kruskall-Wallis test the groups differed in the percentage of time spent at the stop sites relative to the duration of the recorded flight ($p= 0.041$). The Dunn's test revealed that this value was significantly higher for the RNP birds than for the C group ($p<0.05$), but not compared to the LNP pigeons. No difference emerged between the LNP and RNP groups (median percentage of time as follows: C 34.6%, LNP 59.5%, and RNP 76.9%).

Tortuosity. The analysis of the tortuosity applied on sectors of the tracks at increasing distances from the release site showed a significant difference between the three experimental groups (Two way RM ANOVA $F_{2,18}= 5.753$ $p= 0.012$). As shown in Fig. 3, the RNP showed a smaller vector length than both C (Student-Neuman-Kewls method $p=0.010$) and LNP (Student-Neuman-Kewls method $p=0.027$). The analysis revealed a statistically significant difference among the different distances from the release site (two way RM ANOVA $F_{28,496} = 2.478$, $p<0.001$). In fact, all the pigeons, regardless of the experimental group, consistently showed a more tortuous path soon after having left the release site (portion of the tracks included in the 3-4 km sector) than at greater distances (Student-Neuman-Kewls method $p<0.05$). There was no significant interaction between treatment and distance from the release site (Two way RM ANOVA $F_{56,496} = 0.678$, $p= 0.964$).

Efficiency index. The RNP pigeons tended to display longer tracks, and therefore a lower efficiency index, than the other two groups although the Kruskall-Wallis test did not reveal a significant difference between the efficiency index of the whole tracks of the three groups of birds (the median efficiency indices are 0.680, 0.480 and 0.435 for C, LNP and RNP respectively).

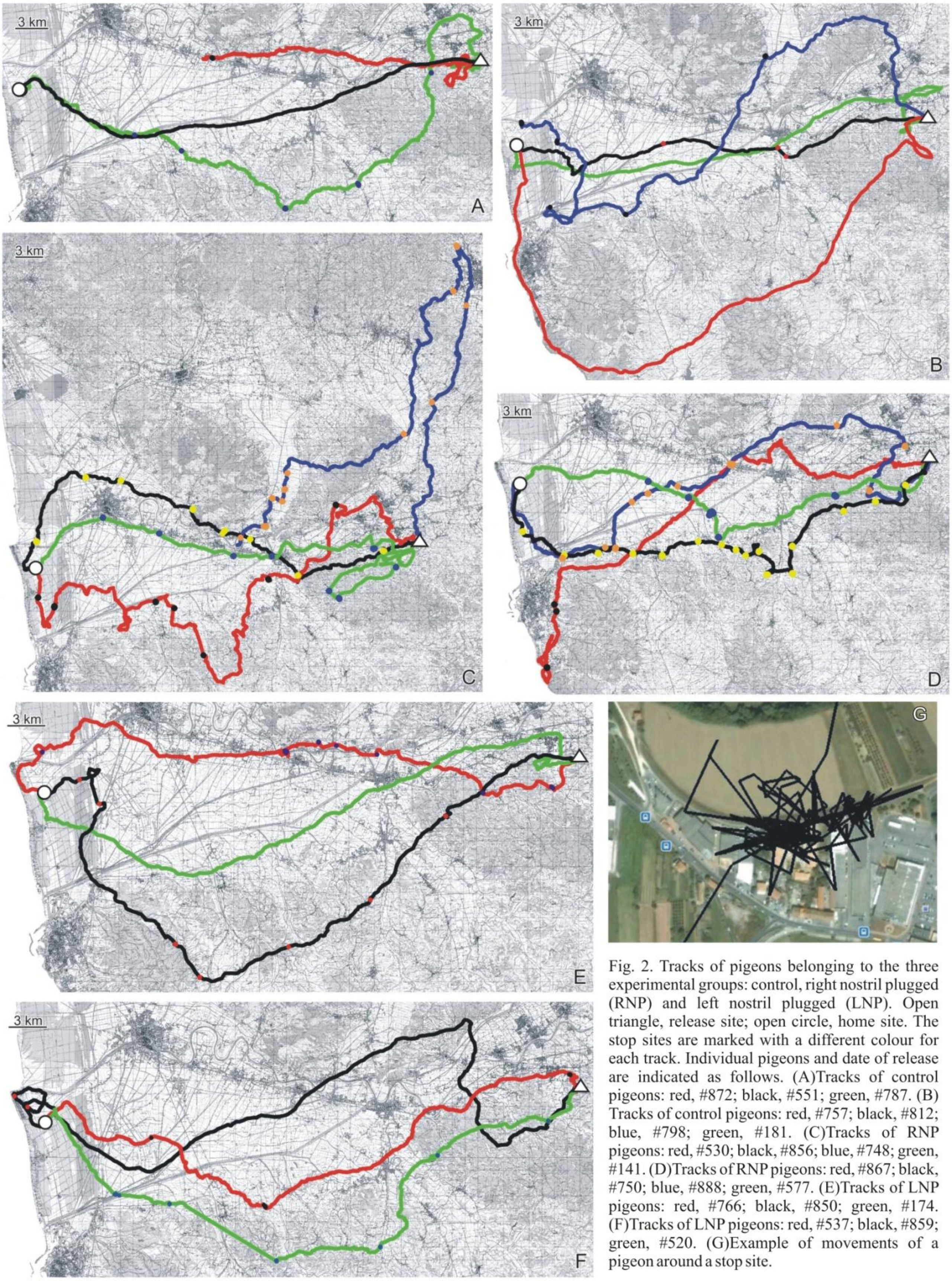

Fig. 2. Tracks of pigeons belonging to the three experimental groups: control, right nostril plugged (RNP) and left nostril plugged (LNP). Open triangle, release site; open circle, home site. The stop sites are marked with a different colour for each track. Individual pigeons and date of release are indicated as follows. (A)Tracks of control pigeons: red, #872; black, #551; green, #787. (B) Tracks of control pigeons: red, #757; black, #812; blue, #798; green, #181. (C)Tracks of RNP pigeons: red, #530; black, #856; blue, #748; green, #141. (D)Tracks of RNP pigeons: red, #867; black, #750; blue, #888; green, #577. (E)Tracks of LNP pigeons: red, #766; black, #850; green, #174. (F)Tracks of LNP pigeons: red, #537; black, #859; green, #520. (G)Example of movements of a pigeon around a stop site.

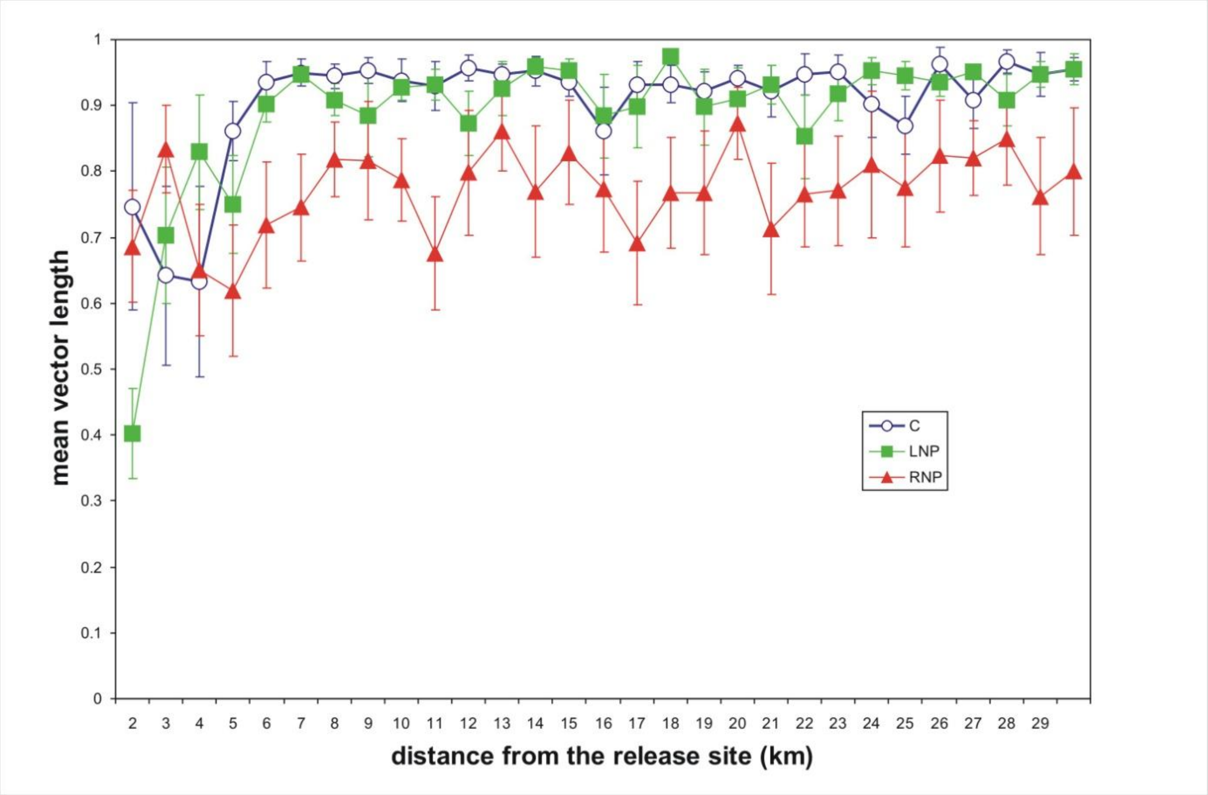

Fig.3. Mean vector lengths relative to the portion(s) of the track falling within each ring as shown in Fig.1, and calculated on the directions taken by the birds moving from one fix to the next. The mean vector length is taken as a measure of the tortuosity of the path.

***Virtual vanishing bearings and virtual vanishing and homing times*.** The three groups of pigeons were significantly oriented at both 1 (Rayleigh test: C p<0.001; LNP p<0.005; RNP p<0.02) and 2 km (Rayleigh test C, p<0.002; LNP, p<0.02; RNP p<0.01) from the release site. The mean vector length and direction, and the homeward component are the following for each group of birds: 1 km from the release site C, 0.95 265° +0.95; LNP 0.85 247° +0.80; RNP, 0.71 243° +0.65; 2 km from the release site C, 0.93 262° +0.92; LNP 0.84 243° +0.77; RNP, 0.77 243° +0.70. The Mardia-Watson-Wheeler test did not reveal any significant difference in orientation between the groups (p>0.5) at both 1 and 2 km from the release site.

The virtual vanishing times (relative to 2 km distance) of the three groups were not significantly different according to the Kruskall-Wallis test (p>0.1; median vanishing times: C 2’24’’; LNP 3’52’’; RNP 2’55’’).

The three experimental groups did not differ in their homing times according to the Kruskall Wallis test (p>0.05) although the RNP group tended to take longer time to home (median homing times: C 3^h 12’; LNP 4^h 02’; RNP 7^h 40’).

DISCUSSION

The analysis of the homing flight path of experienced pigeons receiving a unilateral olfactory input highlighted some of the effects of the right nostril occlusion, which remained undetected when observing only the initial orientation of the birds at vanishing.

Consistent with a previous report on the orientation performance of experienced unilaterally anosmic pigeons (Benvenuti and Gagliardo, 1996), no functional asymmetry emerged in the virtual vanishing bearing, i.e. the direction of the birds at 2km from the release site.

In contrast, the analysis of the tracks revealed that the occlusion of the right nostril apparently affected the behaviour of the birds *en route*. The birds receiving the olfactory input only from the left nostril interrupted their journey more often than both those receiving input from the right olfactory mucosa only and those receiving bilateral olfactory inputs. Therefore, the delayed movement behaviour of the RNP pigeons cannot be explained with an effect of the nasal plug *per se*, but is instead consistent with the interpretation that the treatment specifically affects the right olfactory system. An inspection of the behaviour of the birds at their stop sites revealed that the pigeons did not simply interrupt their homing flight for resting, but also performed some flight activity around the stop site, which might represent an exploratory behaviour for sampling navigational cues. This hypothesis should be testable in the future using data loggers recording even higher-definition GPS data, allowing for a test of directional bias within flights around a stop site. Interestingly, the RNP pigeons exhibited such exploring activity more often compared with the control pigeons, which might suggest that birds have difficulty gathering and processing olfactory cues received with the left olfactory mucosa. Nevertheless, the main behavioural difference between the LNP and RNP birds emerged in the tortuosity of the flight path. In fact, the group released after occlusion of the right nostril displayed a more tortuous flight path than both the un-manipulated control pigeons and the birds smelling with the right nostril. On the whole, the behaviour of the RNP pigeons suggested a specific role of the right nostril in processing olfactory information useful for the operation phase of the navigational map. This might be due to an advantage of the right nostril/olfactory bulb in perceiving and/or discriminating the local environmental odours, although this functional dominance is not accompanied by a higher immediate-early gene ZENK expression in the right olfactory bulb in comparison with the left one (Patzke et al., 2010).

Our findings are consistent with the reported asymmetry in favour of the right nostril observed in chicks discriminating between familiar and unfamiliar olfactory rearing objects

(Vallortigara and Andrew, 1994) or responding with head shaking to a high concentration of eugenol (Rogers et al., 1998). Interestingly, a lateralization in favour of the right nostril has also been demonstrated in humans, particularly in evaluating odour intensity (Thuerauf et al., 2008) and in olfactory detection and discrimination (Kobal et al., 2000; Zatorre and Jones-Gotman, 1990). According to anatomical studies on homing pigeons, the olfactory bulbs project mainly to the piriform cortex through both ipsilateral and contralateral fibres, although the latter are less numerous (Bingman et al., 1994; Reiner and Karten, 1985). A specific role of the piriform cortex in pigeon navigation is supported by lesion experiments, which have shown a navigational impairment in the piriform-cortex-ablated pigeons (Papi and Casini, 1990). The same was observed in ZENK activation experiments, which showed an activation of the piriform cortex following displacement to an unfamiliar location (Patzke et al., 2010). Release experiments on pigeons subjected to unilateral piriform cortex ablation showed that both the left and right portions of the piriform cortex are involved in the homing process from unfamiliar sites, but that the left piriform cortex plays a dominant role. In fact, although the birds with ablation to the left piriform cortex were randomly scattered after release, the pigeons with lesion to the right piriform cortex displayed an unimpaired initial orientation (Gagliardo et al., 2005a). It is worth noting that the pattern of the scattering is reversed when occluding one nostril. In fact, a similar impairment in initial orientation occurs in birds released either after ablation of the left piriform cortex or after occlusion of the right nostril (Gagliardo et al., 2007). Despite the fact that the contralateral contribution of projections from the receptor to the central processing areas (as the piriform cortex) in the olfactory system of birds is low, whereas in the visual system all fibres of the optic nerve cross over completely (Güntürkün, 1997), interestingly, we observed a similar pattern of functional asymmetry, i.e. an advantage of the right receptor and a dominance of the left hemisphere in processing the sensory inputs (Gagliardo et al., 2001; Gagliardo et al., 2005b; Prior, 2006). This suggests a possible involvement of other brain structures and neural connections in olfactory-guided navigation other than the (mainly) ipsilateral projection between the olfactory bulb and the piriform cortex.

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2.2 HOMING PIGEONS ONLY NAVIGATE IN AIR WITH INTACT ENVIRONMENTAL ODOURS: A TEST OF THE OLFACTORY ACTIVATION HYPOTHESIS WITH GPS DATA LOGGERS

ABSTRACT

A large body of evidence has shown that anosmic pigeons are impaired in their navigation. However, the role of odours in navigation is still subject to debate. While according to the *olfactory navigation hypothesis* homing pigeons possess a navigational map based on the distribution of environmental odours, the *olfactory activation hypothesis* proposes that odour perception is only needed to activate a navigational mechanism based on cues of another nature. Here we tested experimentally whether the perception of artificial odours is sufficient to allow pigeons to navigate, as expected from the olfactory activation hypothesis. We transported three groups of pigeons in air-tight containers to release sites 53 and 61 km from home in three different olfactory conditions. The Control group received natural environmental air; both the Pure Air and the Artificial Odour groups received pure air filtered through an active charcoal filter. Only the Artificial Odour group received additional puffs of artificial odours until release. We then released pigeons by recording their tracks with 1Hz GPS data loggers. We also followed non-homing pigeons using an aerial data readout to a Cessna plane, allowing, for the first time, the tracking of non-homing homing pigeons. Within the first hour after release, the pigeons in both the Artificial Odour and the Pure Air group (receiving no environmental odours) showed impaired navigational performances at each release site. Our data provide evidence against an activation role of odours in navigation, and document that pigeons only navigate well when they perceive environmental odours.

INTRODUCTION

Almost four decades ago, Papi and colleagues showed that homing pigeons without the ability to smell have dramatically reduced their navigational abilities (Papi et al., 1971). Since then, a large body of evidence has been collected showing that lesions to the olfactory system consistently produce a disruption of the birds' homing abilities (see for ref. (Wallraff, 2005b; Gagliardo et al., 2006; 2008; 2009a). This phenomenon has been explained by Papi with the olfactory navigation hypothesis (Papi et al., 1972), which predicts that pigeons are able to build up an odour-based navigational map by associating the wind-borne environmental odours with the directions from which they blow at the home area. Once displaced, the pigeons are able to recognise the local prevalent odours characterising the release location, thus determining the direction of displacement. Once the birds know where they are relative to home, they can orient by using a sun or a magnetic compass. Thus in its most general form, the olfactory navigation hypothesis proposes that in pigeons environmental odours are integrated to build up a mental representation of wide geographical areas around the home loft.

However, the olfactory navigation hypothesis is not uncontested (Wiltschko, 1996). Recently, Jorge and colleagues (Jorge et al., 2009b; 2009a) suggested that impaired navigation of anosmic pigeons is due to the fact that olfactory stimuli prime the navigational capabilities of birds. Under this hypothesis, environmental odours are solely needed to activate a navigational system that in turn is based on non-olfactory cues (Jorge et al., 2009b; 2009a). In support of this hypothesis the authors reported that pigeons transported in charcoal-filtered air and released after anaesthesia of the olfactory mucosa displayed scattered initial orientation. In contrast, pigeons transported in pure air, but stimulated with artificial non-sense odours, were not different from control pigeons, irrespective of the fact that the nasal anaesthesia before release prevented them from smelling environmental odours.

However, it is difficult to reconcile the above findings (Jorge et al., 2009b; 2009a) with a large body of previous evidence against a priming role of odours on a non-olfactory navigational system. In particular, experiments testing intact pigeons after manipulations of their housing conditions during map learning contradict the odour-priming hypothesis. Pigeons were unable to develop navigational abilities if raised in aviaries provided with screens preventing the birds from detecting wind directions (Wallraff, 1966; Gagliardo et al., 2001; Odetti et al., 2003b); in contrast, pigeons exposed to the natural winds were able to orient, even if the view of the surroundings was obstructed (Wallraff, 1970). When the directions of the winds at the home loft were deflected (Baldaccini et al., 1975; Waldvogel et al., 1978; Baldaccini et al., 1978) or inverted (Ioalè et al., 1978), a correspondent deflection or inversion of the birds' initial orientation was observed. Other important evidence that odours provide spatial information useful for navigation comes from experiments in which pigeons exposed to artificial odour currents during map learning displayed the expected orientation on the basis of the odour stimuli provided at the release site (Papi et al., 1974; Ioalè et al., 1990). It is worth noting that artificial odour stimuli at the release site determined the expected orientation only if they had been associated by the birds with the artificial wind direction during map learning (Papi et al., 1974; Ioalè et al., 1990), while they acted as disturbing factors if they just represented non-sense odours (Benvenuti et al., 1973; 1977). Other important evidence, supporting a specific role of environmental odour cues in navigation comes from an experiment in which pigeons, transported in air-tight containers ventilated with pure air, were exposed at the release site to air sampled either from an open field or from a thick vegetation area (maize field or forest) before being released after nasal anaesthesia (Wallraff et al., 1992). This experiment showed that the birds exposed to air of the release site sampled from the thick vegetation area were poorer in homeward orientation than the birds allowed to smell the release site air collected in an open field, probably more representative of the specific odour profile of the release site. All these findings are not explained by assuming a priming role of odours in pigeon navigation, but only by accepting a specific role of environmental smells in the proposed navigational map.

So far, the conflicting evidence supporting either a specific role or a priming role of olfactory stimuli in pigeon navigation has come from experiments reporting only the initial orientation data achieved by recording vanishing bearings. With the present experiment we achieve a major advance in our understanding of the role of olfactory stimuli in pigeon navigation by being able to additionally study homing pigeons’ tracks even when the birds do not home. We used newly developed 1Hz-GPS data loggers that allow for a remote readout of the stored data via a 900 MHz data link (Holland et al., 2009), sending data for up to 18 km from the surface to a small Cessna plane in the air.

To conduct this experiment we transported the pigeons, and kept them at the release site, in air-tight containers where they were exposed to three different odour stimuli conditions. Birds were then released after anaesthesia of their olfactory mucosa. One group of birds was allowed to breath environmental air, one group was exposed only to pure air and a third group was exposed to pure air, but stimulated with artificial non-sense odours. From these treatments we derive two exclusive predictions: i) the olfactory navigation hypothesis is rejected, the olfactory priming hypothesis is accepted: under this scenario both control pigeons and those exposed to ‘priming’ odours will navigate equally well. We thus predict unimpaired navigational performances in both of these groups, but not the group that lacks exposure to any environmental odours during transport and before release. Alternatively, ii) the olfactory navigation hypothesis is accepted and the olfactory priming hypothesis is rejected: here we predict that only the control birds show unimpaired navigational abilities while both other groups that are deprived of environmental odours are impaired. Our data support the latter scenario and thus provide evidence for the olfactory navigation hypothesis.

MATERIALS AND METHODS

General procedure

Thirty-six inexperienced pigeons, about 15-18 months of age and hatched at the Arnino field station (latitude 43° 39’ 26’’ N; longitude 10° 18’ 14’’ E), Pisa, Italy, were used in the study. The pigeons were raised as free flyers and were kept and manipulated according to Italian law on animal welfare.

Twenty days prior to the experimental releases all the birds were equipped with a PVC dummy weight, similar in dimension and weight to the GPS data logger they would be carrying, in order to accustom them to flying with a load. A few days before the experimental tests, the pigeons had been released in a group from different directions up to 7 km from Arnino.

For the test releases we transported three groups of pigeons and kept them at the release site in air tight containers ventilated by aspirators. The Control (C) group container was ventilated by environmental air; both the Pure Air (PA) group and the Artificial Odour (AO) group containers were ventilated by pure air filtered through an active charcoal filter; in addition the Artificial Odour group received different puffs of odours of plant origin (eucalyptus, orange, jasmine, rose, lavender) both during transportation and at the release site about each 20 minutes for the duration of the whole experiment. The administration of the odour puffs was given injecting a 50 ml volume of air saturated with one odour, in the flux of air coming from the charcoal filter to the air tight container. The release experiment started two hours after we had arrived at the release site.

GPS data loggers

We used two different kinds of miniature GPS data loggers storing one position fix every second: for twenty of the tests on C birds we used the loggers by Technosmart (www.technosmart.eu, Rome, Italy); for the other release tests we used loggers by E-obs (www.e-obs.de, Munich, Germany), which also feature remote UHF data download capabilities. The latter device thus allowed aerial data downloads (from a small Cessna airplane, the ‘Spirit of MaxCine’) of pigeons that did not home. The positional fixes stored by a GPS data logger include latitude, longitude, and time of recording. The tracks for each pigeon were uploaded, automatically checked for potential GPS-errors and duplicates, and then stored and made publicly accessible in Movebank (Wikelski, 2010) (www.movebank.org) for each recorded release. Data were then exported from Movebank and were visualised with Google Earth (Google Inc, US).

Test releases

Eleven C, 8 PA pigeons and 8 AO birds were released from Bolgheri (Long. 10° 34’ 35’’ Lat. 43° 13’ 07’’; home direction 337°, home distance 53 km). Eleven C pigeons, 7 PA birds and 7 AO pigeons were released at Montespertoli (Long. 11° 03’ 59’’ Lat. 43° 39’ 09’’; home direction 270°, home distance 61 km). Before the release each pigeon was subjected to anaesthesia of the olfactory mucosa with a single dose of Xylocaine® sprayed through the choanae and then equipped with a GPS data logger. Each pigeon was released singly. The releases took place under sunny conditions, with no or light wind. The homing time on the day of the release was recorded by an observer at the home loft.

Quantitative analyses and statistical procedures

Because the effect of the local anaesthesia of the olfactory mucosa decreases over time (Wallraff, 1988), we analysed separately the sections of the tracks from take-off until one, two and three hours after release, respectively. For the analysis we considered the directions taken by the bird while moving from one point to the next at a speed higher than 5 km/h. We thus calculated the individual mean vector and the relative homeward component. The mean vector distributions relative to the section of the tracks recorded in the first hour, in the first two hours and three hours of each experimental group at both sites were tested for randomness with the one sample Hotelling test (Batschelet, 1981). We also calculated the homeward component (hc) of the second order mean vectors.

Focusing on the first hour after release, we performed a statistical analysis by pooling the data from the two release sites. We considered all tracks of the birds released once, plus the first track suitable for the analysis of the birds released twice. We assessed the performances of the three experimental groups by considering the following parameters, which have been compared with the Kruskall Wallis test: the tortuosity of the track expressed by the individual mean vector length, the orientation of the track expressed by the homeward component of the mean vector, and the efficiency index of the flight. The efficiency index for each pigeon is defined as the beeline between the release site and the point where the bird was after 1 hour from release, divided by the length of the flight path.

Furthermore, to enable comparisons with previously published data, we recorded the virtual vanishing bearings, defined as the directions with respect to the release site of the birds at a distance of 2 km from it. If a pigeon did nothing but circle around the release site at a distance closer than 2 km, it was excluded from the analysis. The circular distributions were tested for randomness by means of both the Rayleigh and V test and compared with the Mardia-Watson-Wheeler test (Batschelet, 1981).

As the birds sometimes stopped (i.e., either landed or moved at a speed lower than 5 km/h) during their homing journey, we calculated the percentage of time the bird was flying in the track recorded in the first hour after release. In addition, we calculated how far the birds were from home at the end of their first hour track section and we recorded the birds homing performances. We compared the percentage of time in flight, the distances at which the birds were after 1 hour and the homing performance of the three groups with the Kruskall-Wallis test. All pigeons we released were included in the analysis of the homing performances. The Dunn's test was used for multiple comparisons.

RESULTS

Some of the Technosmart GPS data loggers used on control pigeons did not record or only started to record a few hours after release and were therefore excluded from the analysis on orientation. For this reason we had to include some additional birds in the control group. Thus the number of pigeons belonging to the C groups was higher, but we obtained a lower number of tracks suitable for analysis.

Details on the performances of Control, PA and AO pigeons obtained in both the release from Bolgheri (North) and Montespertoli (east) are reported in Table 1, 2 and 3, respectively. Examples of tracks of Controls, PA and AO are visualised in Figs. 1-6, which show the portion of the route flown during the first (yellow), the second (red), the third (green), and following hours (blue). All the tracks can be inspected in Movebank (www.movebank.org).

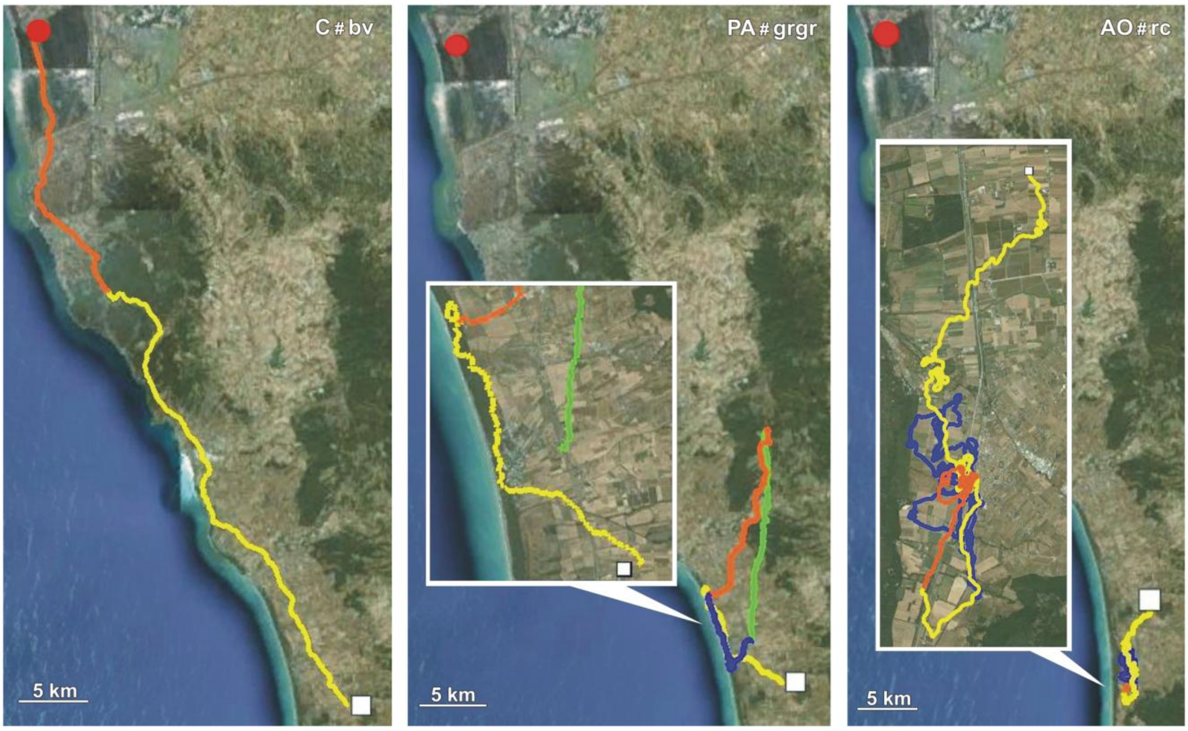

Fig. 1 Examples of tracks: the code of the track and the experimental group is reported on each figure. The red circle and white square represent home and release sites (Bolgheri South and Montespertoli East from home), respectively. The yellow, red, green and blue lines: paths flown during the first, second, third and subsequent hours from release. Triangles indicate that the bird stopped for an entire hour. The colour of the triangle indicates the time range in which the bird stopped.

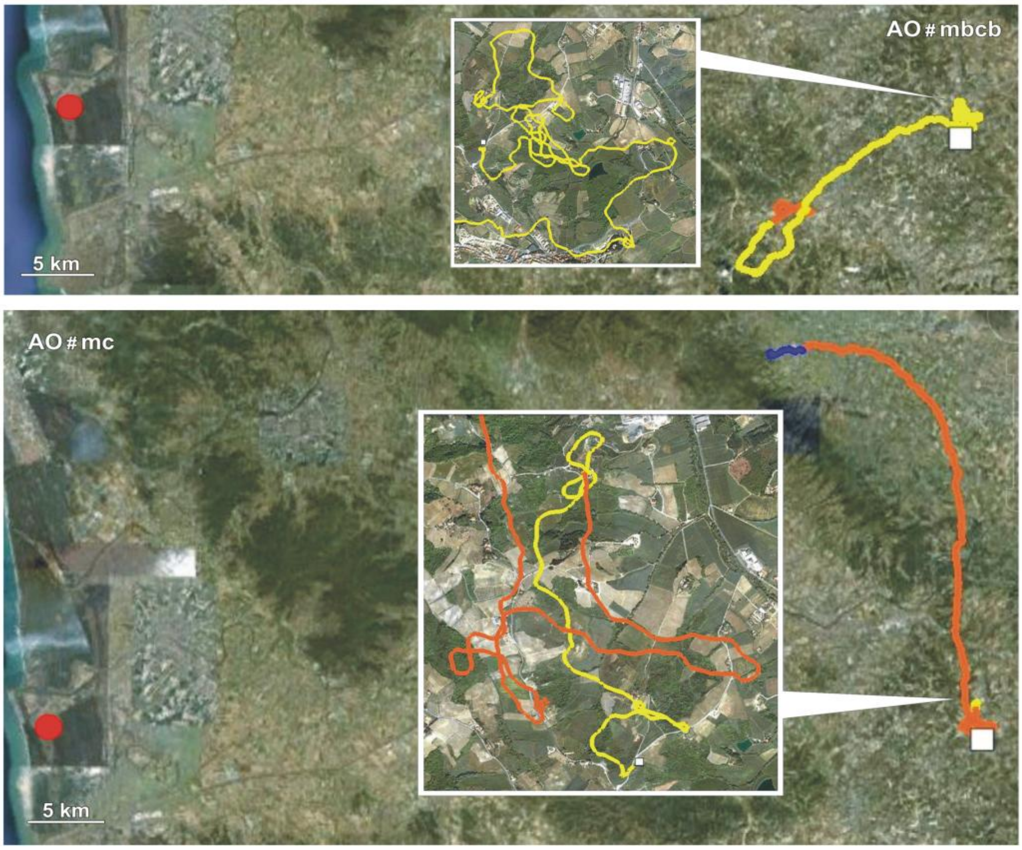

Fig. 2 Examples of tracks. Other explanations as in Fig. 1.

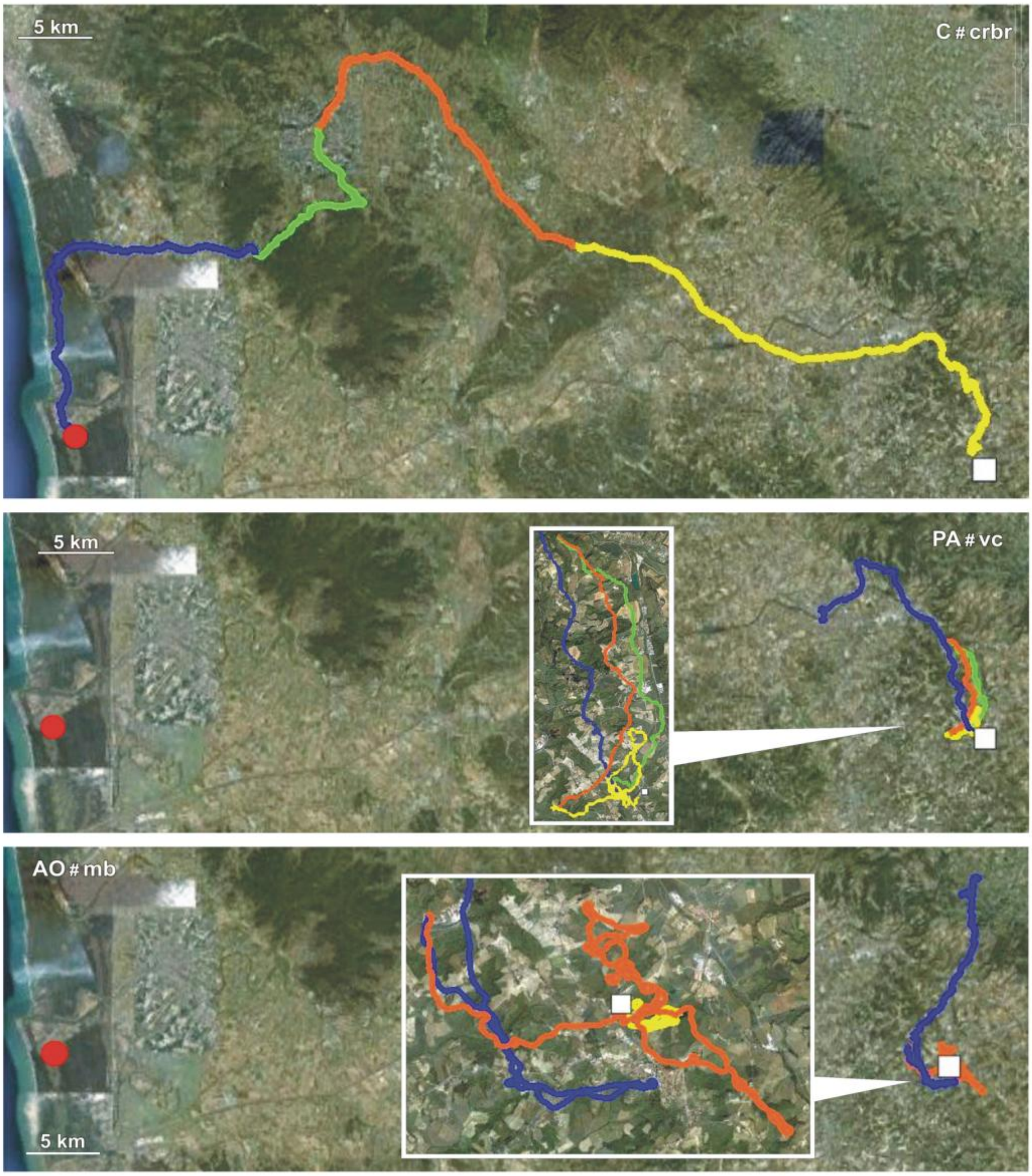

Fig. 3 Examples of tracks. Other explanations as in Fig. 1

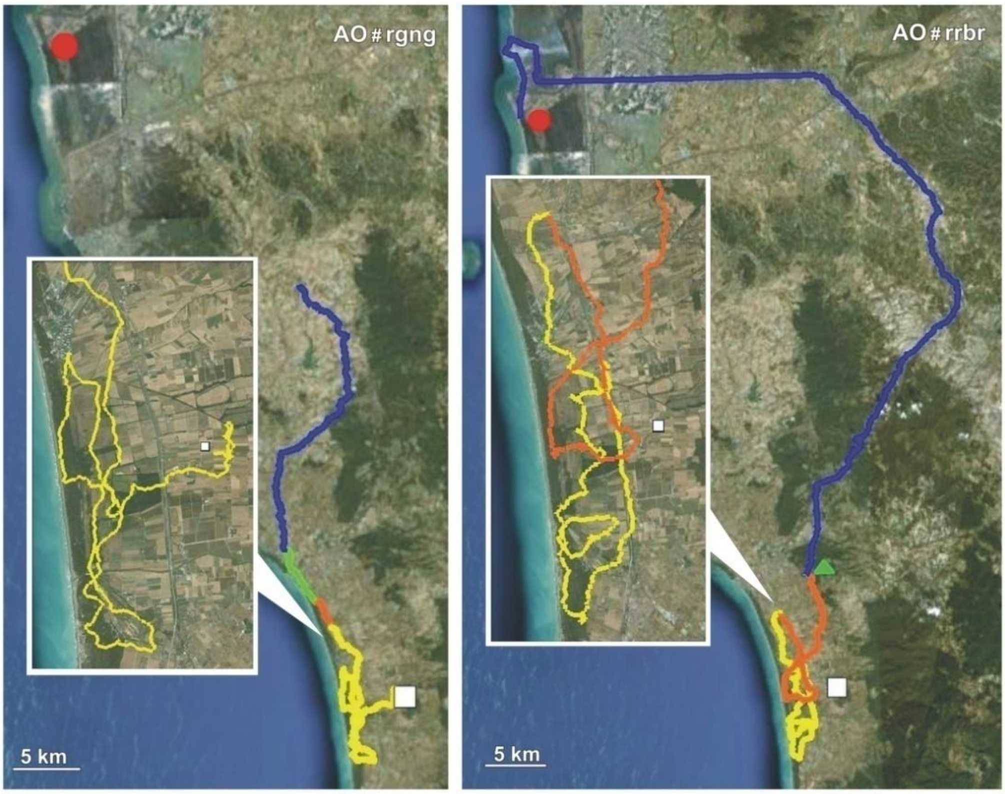

Fig. 4 Examples of tracks. Other explanations as in Fig. 1.

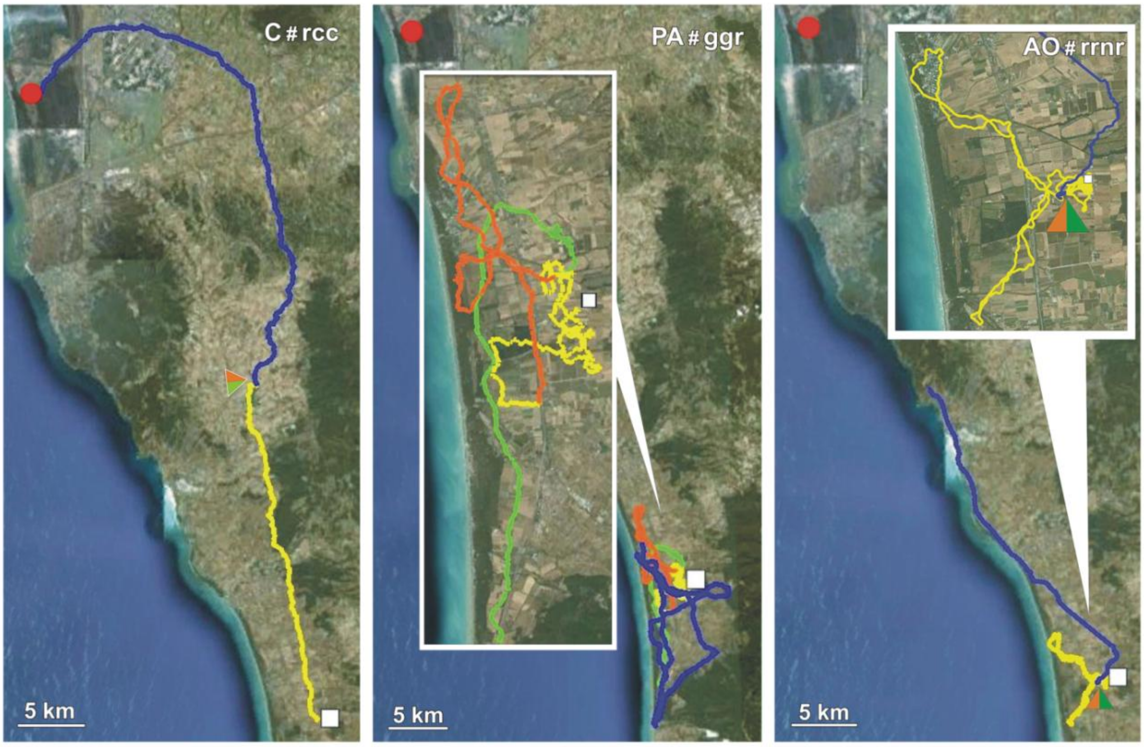

Fig. 5 Examples of tracks. Other explanations as in Fig. 1.

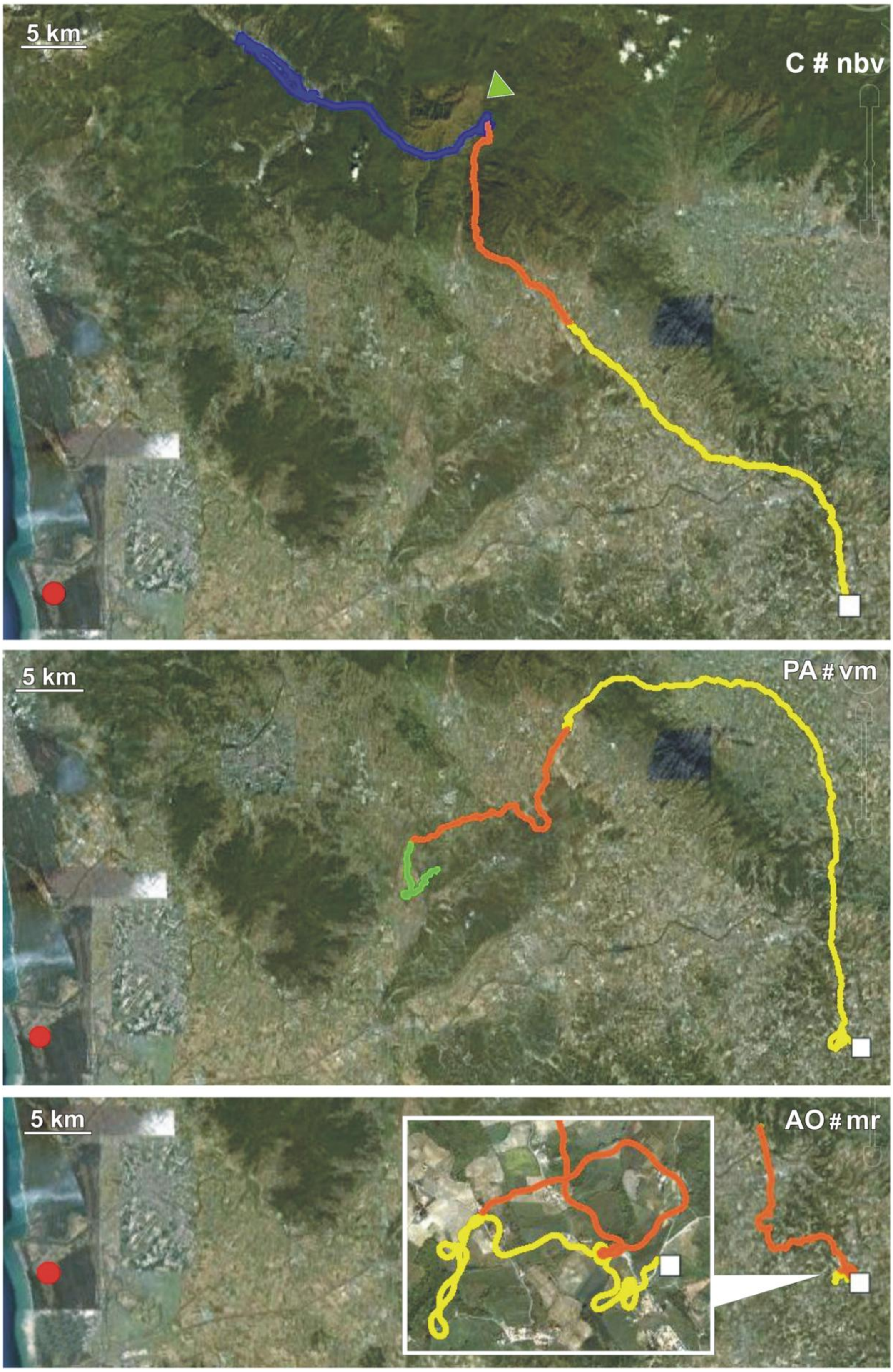

Fig. 6 Examples of tracks. Other explanations as in Fig. 1.

Considering the portions of the tracks recorded within the first three hours after release (Fig. 7) we observed that the individual mean vector distributions were significantly different from random at both release sites for both C (Hotelling test, Bolgheri: $p<0.05$, $T_{(2,5)}=$ 26.45435; Montespertoli: $p<0.05$ $T_{(2,3)}=$ 72.19185) and PA (Hotelling test, Bolgheri: $p<0.001$, $T_{(2,6)}=98.762$; Montespertoli: $p<0.05$ $T_{(2,5)}=$ 15.9391). The AO pigeons' mean vector distribution is randomly scattered at Bolgheri (Hotelling test, $p>0.05$ $T_{(2,6)}=3.58884$) and significantly different from uniform at Montespertoli ($p<0.05$ $T_{(2,5)}=$ 27.68512). If we restrict the analysis to the portions of the tracks recorded within the first two hours after release (Fig. 8) the pigeons' distributions of both C (Hotelling test, Bolgheri: $p<0.01$, $T_{(2,5)}=$ 33.22055; Montespertoli: $p<0.05$ $T_{(2,3)}=$ 55.34831) and PA (Hotelling test, Bolgheri: $p<0.05$, $T_{(2,6)}=24.73326$; Montespertoli: $p<0.05$ $T_{(2,5)}=$ 30.00647) are still significantly oriented at both sites. By contrast, the AO birds' mean vector distributions are not significantly different from random at both sites (Hotelling test, Bolgheri: $p>0.05$ $T_{(2,6)}=3.268577$; Montespertoli: $p>0.05$ $T_{(2,5)}=$ 9.930811). The observation of the behaviour of the birds within the first hour after release (Fig. 9) revealed that the C birds displayed mean vector distributions different from random at both sites (Hotelling test, Bolgheri: $p<0.01$, $T_{(2,5)}=$ 71.43776; Montespertoli: $p<0.05$ $T_{(2,3)}=$ 68.66537), the PA birds were randomly scattered at both sites (Hotelling test, Bolgheri: $p>0.05$, $T_{(2,6)}=8.58531$; Montespertoli: $p>0.05$ $T_{(2,5)}=$ 10.38986), and the AO pigeons turned out to be randomly scattered at Bolgheri (Hotelling test, $p>0.05$ $T_{(2,6)}=0.4150136$); and significantly oriented at Montespertoli (Hotelling test, $p<0.05$ $T_{(2,5)}=$ 14.82878). However, the AO birds, although significantly oriented, showed a very small homeward component (see Fig. 9 for details).

As the nasal anaesthesia decreases over time, a detailed analysis of the section of the tracks recorded in the first hour after release is reported below.

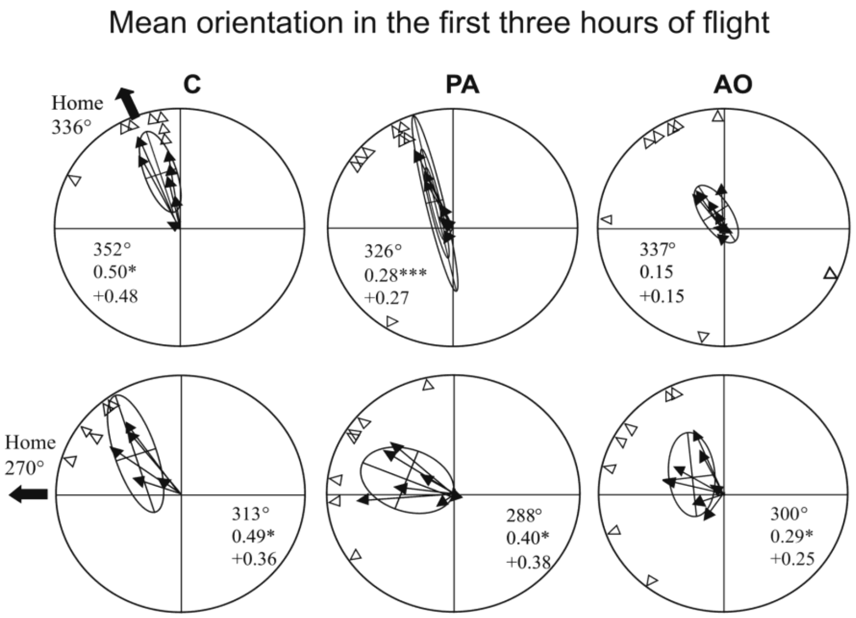

Fig. 7 Mean vector distributions relative to the sections of the tracks recorded in the first three hours after release from Bolgheri (home direction 336°) and Montespertoli (home direction 270°), respectively. Outer arrows: home direction. Inner arrows: individual mean vectors (see Material and methods for further explanations). The open triangles at the periphery of the circle represent the directions of the individual mean vectors. Confidence ellipses of the distributions are reported. The second order mean vector lengths and directions are reported inside the circles. Asterisks indicate the significance level of the one sample Hotelling test: * p<0.05; ** p<0.01; *** p<0.001

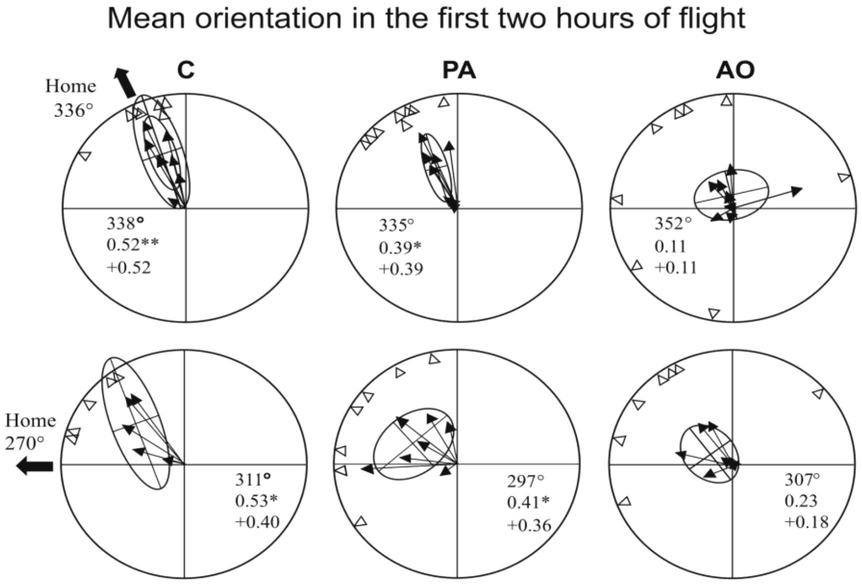

Fig. 8 Mean vector distributions relative to the sections of the tracks recorded in the first two hours after release. Further explanations as in Fig. 7.

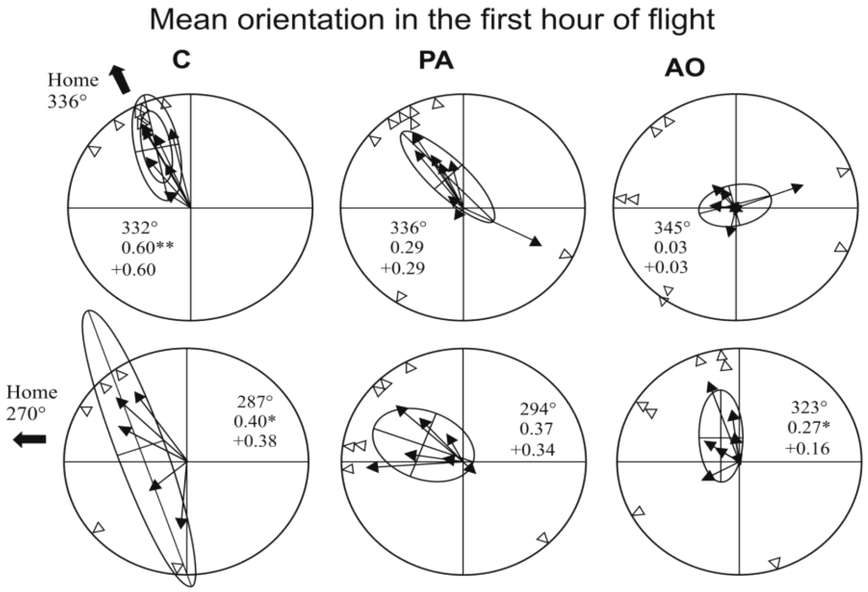

Fig. 9 Mean vector distributions relative to the sections of the tracks recorded in the first hour after release. Further explanations as in Fig. 7.

Efficiency index.

The statistical analysis of the pooled efficiency indexes relative to the data from both sites (Fig. 10) resulted in a significant difference between groups (Kruskall-Wallis test, p<0.05). In particular, the control group displayed an efficiency index significantly higher with respect to the AO birds (Dunn's test, C vs. AO p<0.01). No differences emerged between the PA pigeons and both the other two experimental groups.

Tortuosity and homeward component

The statistical analysis of tortuosity (Fig 11) and homeward component (Fig. 12), done on the pooled data from both sites, revealed a significant difference between the groups (Kruskall-Wallis test: tortuosity p<0.01; homeward component p<0.05). In particular the AO pigeons displayed a more tortuous path (Dunn's test p<0.01, median= 0.24) and a lower homeward component (p<0.05, median = +0.08) than the C pigeons (median tortuosity = 0.69, median hc =. +0.48). The PA birds were not significantly different from the other two groups both in tortuosity (median = 0.46) and homeward component (median = +0.42).

Flight time

The statistical analysis on the percentage of time the birds spent flying during the first hour from release did not highlight any difference between groups (Kruskall-Wallis test, Bolgheri p=0.054; Montespertoli p>0.05) although the AO pigeons tended to stop longer (median percentage of time in flight: Bolgheri C 80%, PA 48%, AO 29%; Montespertoli C 65%, PA 52%, AO 18%).

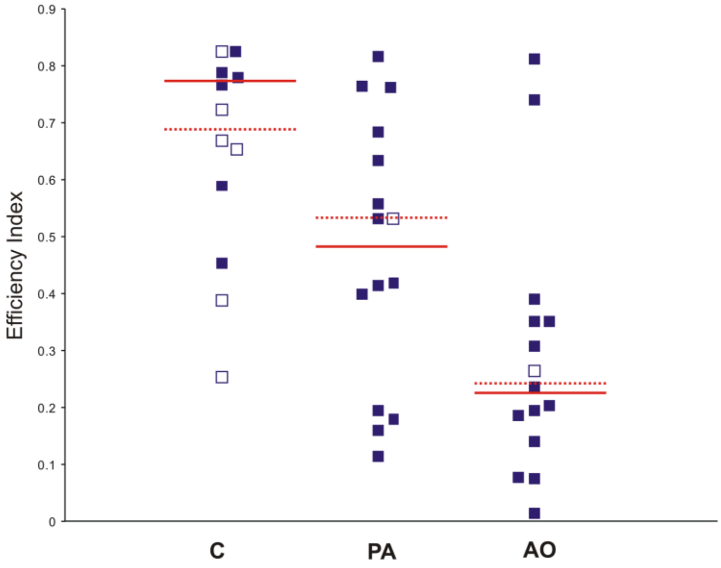

Fig. 10. Pooled efficiency index of the section of the track recorded in the first hour after release. The filled and open symbols represent the data included and not included in the pooled analysis respectively. The median values of the data included in the pooled analysis are indicated by the unbroken lines. The dotted lines indicate the median values of the whole data set.

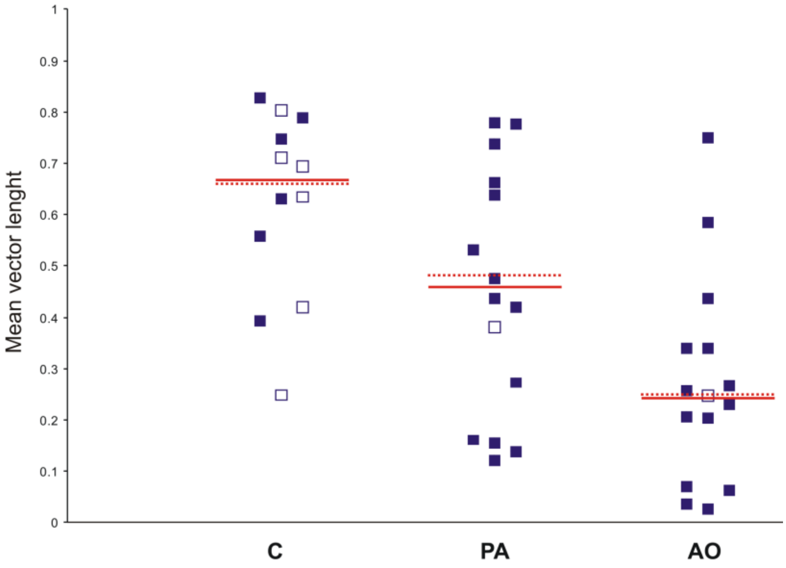

Fig. 11. Tortuosity of the sections of the tracks recorded in the first hour after release, expressed by the mean vector lengths (see Materials and methods for further explanations). Other explanations as in Fig. 10.

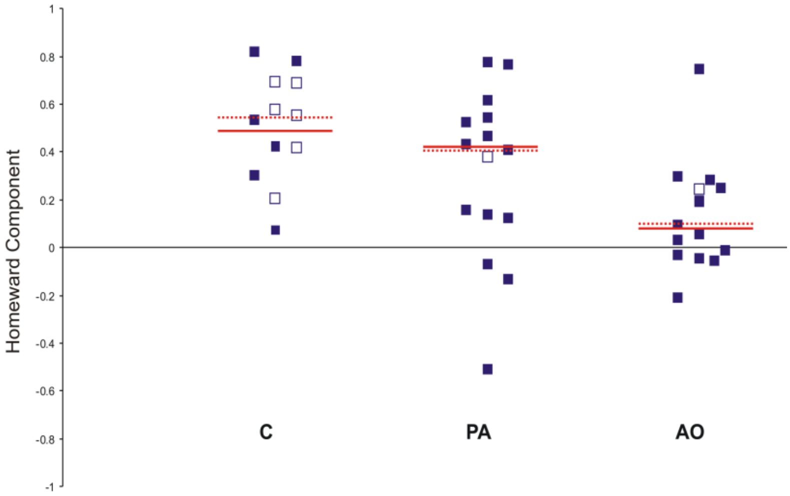

Fig. 12. Homeward component of the sections of the tracks recorded in the first hour after release. Other explanations as in Fig. 10.

Virtual vanishing bearings

The virtual vanishing bearing distributions are shown in Fig. 13. At Bolgheri the virtual vanishing bearings distribution of the C pigeons (mean vector length and direction: n= 7 r = 0.79 α = 295° hc = +0.59) was significantly different from random both according to the Rayleigh ($p<0.01$) and V test ($p<0.05$). The PA group displayed a virtual vanishing bearing distribution (n = 8 r = 0.70 α = 276° hc=+0.34) different from random according to the Rayleigh test ($p<0.02$), but not according to the V test ($p>0.05$), which takes into account the orientation towards the expected direction. The AO pigeons displayed an impaired initial orientation performance (n = 7 r = 0.61 α = 268° hc=+0.22) as their virtual vanishing distribution was not different from random according to both the Rayleigh and V test ($p>0.05$). At Montespertoli the C pigeons' virtual vanishing bearing distribution (n= 5 r = 0.74 α = 320° hc = +0.48) was not significantly different from random both according to the Rayleigh (p= 0.054) and V test ($p>0.05$). At this release site both PA (n= 7 r = 0.78 α = 316° hc = +0.55) and AO pigeons (n= 6 r = 0.76 α = 321° hc = +0.50) displayed significantly oriented virtual vanishing bearing distributions (Rayleigh test: $p<0.01$, V test: $p<0.05$).

Homing performance

Considering the last position of the birds recorded within the first hour (Fig. 14), the Kruskall Wallis test revealed a significant difference in the distance from home between the groups (p=0.012) in the release from Bolgheri (home distance 53 km). In particular the C birds were significantly closer to home than the AO pigeons (Dunn's test $p<0.01$; median distance from home in km: C 29.9; PA 43.8, AO 53.5). In the release from Montespertoli the GPS of the C bird #cgr stopped recording after 20 minutes from release. Therefore we considered in the analysis the last recorded position in the calculation of the distance from home. The distance from home in the first hour from release did not differ significantly (Kruskall Wallis p=0.187) among the birds released at Montespertoli (home distance 61 km), although the same trend in the values of the median distance from home occurred (C 55.9; PA 55,5; AO 61). Looking at the individual performances, it is worth noting that the AO birds were consistently in the vicinity of the release site for both release experiments one hour after release. The three groups of pigeons were significantly different in their homing performances when released at Bolgheri (Kruskall-Wallis test, $p<0.005$). In fact, the C pigeons (median homing speed 7.97 km/h) were significantly faster at homing than both PA (half of the PA birds homed later than the day of release; Dunn's test: C vs. PA $p<0.01$) and AO (more than half of the AO birds homed the day after the test; Dunn's test C vs. AO $p<0.05$). In contrast, no statistical difference between groups emerged at Montespertoli (more than half of both the C and PA birds homed within the next day of the test; more than half of the AO birds did not home or homed later than the day after the test).

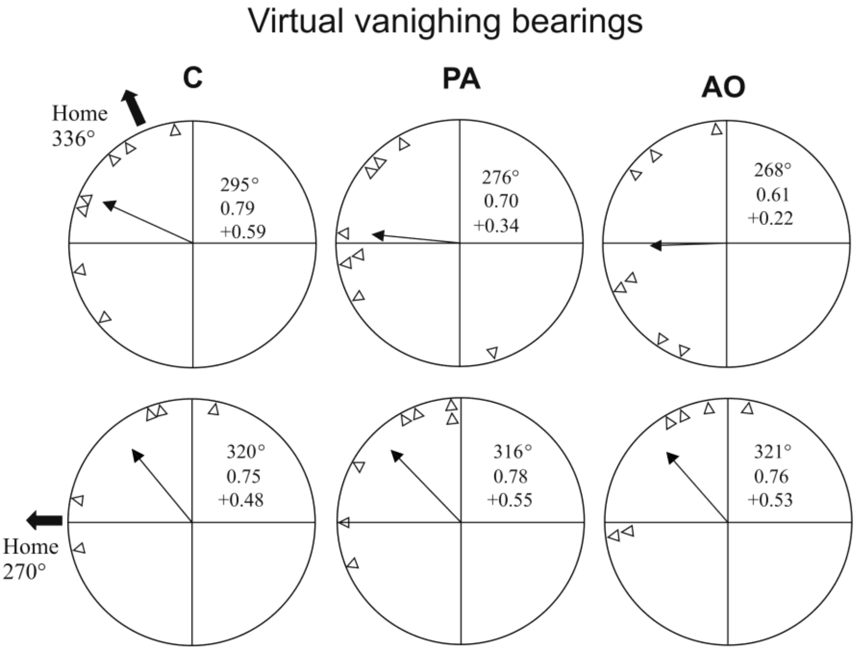

Fig. 13 Virtual vanishing bearings. Triangles inside the diagram represent the orientation of each bird at 2 km from the release site. Outer arrows: home direction. Inner arrows: mean vector of the distribution. Mean vector length and direction, and homeward component are reported (see Results for further explanations).

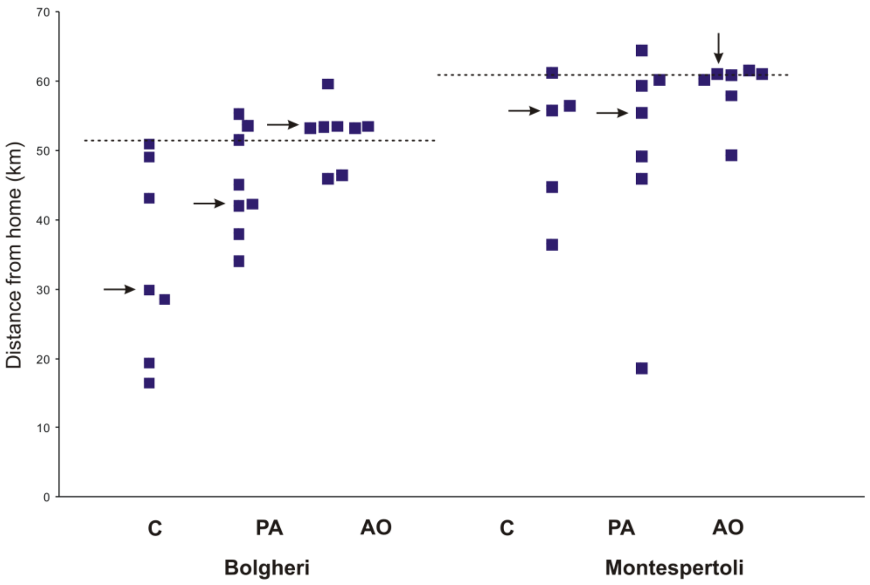

Fig. 14 Distance from home. Distance of the birds from home recorded 1 hour after release. Broken line: distance of the release site from home. The arrows indicate the median value of the group.

The use of the GPS logger that allowed for remote downloading from a plane avoided the selection of the best performing subjects to be included in the analysis and gave us information about the behaviour of non-homed birds. In the case of the three lost control pigeons we were able to document at least the initial track of one of them (#cgr) and the position of another subject which was localised near the home area (#cm) after a few hours from release. The lost PA pigeon (#vbcb) oriented towards North in the first two hours after release, while in the third hour corrected its orientation towards the home direction. All the lost AO birds (#mv, #mr, #rgcg) remained near the release site even in the third hour after release.

DISCUSSION

The olfactory navigation hypothesis attributes a specific role of olfactory environmental cues in pigeons' navigation. On the contrary, the olfactory activation hypothesis explains the impaired performances of anosmic pigeons largely documented by experiments conducted over forty years (see Wallraff, 2005a) by assuming that odour perception is needed to activate a navigational mechanism based on non-olfactory cues. According to the olfactory activation hypothesis, pigeons prevented from smelling environmental odours during transportation, and released under nasal anaesthesia should display unimpaired navigational performances, provided that they had been stimulated by artificial odours before release. The data reported in this experiment are consistent with the olfactory navigation hypothesis and contradict the olfactory activation hypothesis.

As the effect of nasal anaesthesia decreases over time, the analysis of movement behaviour restricted to the first hour after release is the most informative about the effects of the olfactory experience prior to release. Considering the sections of the tracks recorded during the first hour of flight, it emerges that the pigeons exposed to artificial odours before being subjected to nasal anaesthesia and then released, displayed tortuous flight paths with poor homeward orientation. This was similar to what we observed for the birds transported in pure air without additional olfactory stimulation. The behaviour of both the PA and, more importantly, the AO groups clearly differs from that of the control pigeons, which were exposed to environmental olfactory information during transportation and at the release site before receiving nasal anaesthesia. In fact, the C birds displayed significantly oriented mean vector distributions at both sites, with a second order mean vector direction close to the home direction; the PA pigeons' mean vector distributions were not different from random at both sites and the AO pigeons displayed a scattered mean vector distribution at Bolgheri, and a significantly oriented distribution at Montespertoli, but with a small homeward component. In addition, the birds stimulated with artificial odours turned out to circle in the release site area without gaining distance from it. In fact, their efficiency index, calculated on the section of the track recorded on the first hour after release, was significantly lower in comparison to that of the control pigeons. Also this aspect of their behaviour contradicts the expectations predicted by the olfactory activation hypothesis.

On the whole, the PA pigeons tended to show performances intermediate between those of the control birds and the birds stimulated with artificial odours for all the parameters considered. A visual inspection of the tracks revealed that some of the PA pigeons displayed homeward orientation already within the first hour after release. Moreover, if we consider the first two and the first three hours after release, the tendency of the PA pigeons to recover their navigational abilities over time becomes evident. In contrast, the performance of the AO pigeons turned out to be impaired also after including in the analysis the portions of the tracks recorded after the effect of anaesthesia had probably ceased. In fact, the mean vector distributions of the AO pigeons were not significantly different from random, even when considering the portions of the tracks recorded in the first two (both at Bolgheri and Montespertoli) or in the first three hours (at Bolgheri) after release. Therefore the exposition to the artificial odours seemed to have an even more dramatic effect on the abilities of the birds to head home than just the permanence in pure air. To explain this fact it is well worth discussing the efficacy of nasal anaesthesia on the olfactory sense. It was shown previously using cardiac acceleration recording in response to odour stimuli that nasal anaesthesia delivered both through the nostrils (Wallraff, 1988) or the choanae (Schmidt-Koenig and Phillips, 1978) largely abolished the pigeons' sensitivity to smells. However, the effect decreases over time starting from 15 minutes after the treatment, and it is quite variable across individuals also because it proved difficult to standardise the administration of the spray (Wallraff, 1988). In particular, the treatment through choanae, as in the present paper, seems to abolish the olfactory sensitivity for about one hour. The variable and temporal efficacy of the anaesthesia of the mucosae is consistent with the pattern observed in the PA pigeons, and this might explain the good performances of some of the PA birds even in the first hour of flight. It is worth noting that the homing experiments in which birds were made anosmic with nasal anaesthesia have produced variable results, while it has been consistently demonstrated that pigeons made anosmic with long lasting methods (zinc-sulphate treatment of the olfactory mucosae or olfactory nerve section) consistently produced a dramatic navigational impairment (Wallraff, 2005b). Differently from the PA, the AO pigeons seemed to be consistently impaired and confused for a longer time than that expected on the basis of the duration of the anaesthesia. One possibility is that when the anaesthesia had ceased the AO pigeons started to smell the artificial odours that were likely to be impregnated in their feathers during their permanence in the air-tight containers. Therefore the perception of the artificial odours might have prevented the perception and/or the recognition of the environmental odours. Another possible explanation is that the stimulation with artificial odours during transportation and at the test site before the release might have induced the birds to look for the same kind of stimuli in the environment once the olfactory perception was recovered. A third explanation is that the olfactory receptors intensively stimulated with concentrations of odours much higher than the natural environmental smells are not able to detect very diluted odours, such as those contained in the environment, for sometime. Whatever the explanation might be, our data are consistent with observations by Benvenuti et al. (Benvenuti et al., 1977; Wallraff et al., 1992), that the stimulation with artificial non-sense odours at the release site acted as a disturbing factor and induced an impairment in pigeons' initial orientation and homing times. The AO birds therefore received a double manipulation: the lack of exposition to environmental air during transportation and at the release site, and exposition to artificial odours masking and confounding the perception of the natural odours. This can plausibly explain the consistently poor navigational performances of the AO group.

The specific role of environmental odours in pigeon navigation has been demonstrated in a variety of experiments on intact birds when wind-born odours were manipulated at the home loft (Wallraff, 2005a). All these data are consistent with the olfactory navigation hypothesis, but contradict the olfactory activation hypothesis. Along this line of argument it is worthwhile discussing the overall plausibility of the olfactory activation hypothesis. Among all the sensory manipulations (Wallraff, 2005a) applied on homing pigeons released at unfamiliar locations, only olfactory manipulations consistently produced a homing impairment in the treated birds. To accept the olfactory activation hypothesis one should therefore admit that pigeons are never impaired at homing after a direct manipulation of their navigational mechanism, but only after the manipulation of the system (the olfactory system) activating it. We consider this implausible and in open contrast to the principle of parsimony.

Both our current GPS tracking data including non-homing homing pigeons for the first time, as well as the vast amount of historical initial orientation evidence on the effect of olfactory manipulation in pigeons (Wallraff, 1966; 1970; Gagliardo et al., 2001; Odetti et al., 2003a; Baldaccini et al., 1975; 1978; Waldvogel et al., 1978; Papi et al., 1974; Benvenuti et al., 1973; 1977), contradict the interpretation of the data reported by Jorge et al (Jorge et al., 2009b; 2009a). We could not detect an activational role of olfactory information in pigeon navigation. The inconsistencies of Jorge et al.'s studies with previous data might partly be explained by variability in the effect of the nasal anaesthesia, and partly by the noise inherent in the vanishing bearings data. Interestingly, in our experiment the analysis of the virtual vanishing bearings distribution did not fully correspond with the pigeons' behaviour "en route". Specifically, the virtual vanishing bearing results were consistent with what we observed from the tracks in the release at Bolgheri, but not at Montespertoli. Nevertheless we are convinced that the observation of vanishing bearings is an important method, which becomes highly reliable and meaningful in those cases in which the initial orientation data can be interpreted considering also the homing performance of the released birds. This condition is encountered when the effect of a long lasting manipulation is tested, which is not the case when applying nasal anaesthesia. Therefore GPS tracking of both homing and non-homing individuals can be critically important for resolving controversial aspects on birds navigation (Gagliardo et al., 2009b; 2011), or for highlighting phenomena not detectable by observing the birds' initial orientation (Gagliardo et al., 2011).

In conclusion, consistently to what was reported in previous studies, the results of our experiment constitute further evidence against the activational role of odours in birds' navigation. Instead, our data provide a new piece of evidence for a specific role of olfactory information in the navigational map mechanism in homing pigeons.

TABLES.

RS	Track	Date	r (1)	α (1)	r (2)	α (2)	r (3)	α (3)	ht	at
Bolgheri 336° 53 km	#bb^{1}	02/07/10	0.56	321°	0.51	334°	0.66	351°	7^{h} 55’	A, P
	#bc^{2}	02/07/10	0.79	331°	0.31	350°	0.28	351°	day after	A, P
	#bv^{3}	02/07/10	0.83	332°	0.83	336°	0.83	336°	2^{h} 15’	A, P
	#bgbg^{4}	05/07/10	-	-	-	-	-	-	day after	NR
	#brgr^{5}	05/07/10	-	-	-	-	-	-	6^{h} 25’	I
	#br^{6}	05/07/10	-	-	-	-	-	-	8^{h} 45’	I
	#rmb^{7}	10/07/10	0.42	333°	0.68	331°	0.69	332°	2^{h} 42’	A
	#rcb^{8}	10/07/10	0.25	303°	0.17	300°	0.11	297°	6^{h} 02’	A
	#rcc^{9}	10/07/10	0.71	347°	0.70	347°	0.55	351°	6^{h} 39’	A
	#rvc^{10}	10/07/10	-	-	-	-	-	-	day after	I
	#rvgr^{11}	10/07/10	0.69	336°	0.48	346°	0.45	346°	4^{h} 35’	A
Montespertoli 270° 61 km	#cgr^{12}	07/07/10	0.75	325°	0.75	325°	0.75	325°	lost	A, P
	#cb^{8}	07/07/10	0.40	231°	0.19	284°	0.23	305°	later	A, P
	#crbr^{13}	07/07/10	0.64	299°	0.61	305°	0.48	305°	4^{h} 45’	A
	#cc^{9}	07/07/10	0.63	187°	0.44	287°	0.40	288°	day after	A, P
	#cm^{14}	07/07/10	-	-	-	-	-	-	lost	I
	#cr^{6}	07/07/10	-	-	-	-	-	-	day after	NR
	#cbcb^{15}	07/07/10	-	-	-	-	-	-	lost	NR
	#nbb^{1}	21/07/10	-	-	-	-	-	-	9^{h} 16’	I
	#nrg^{16}	21/07/10	-	-	-	-	-	-	6^{h} 00’	NR
	#nrgng1	21/07/10	-	-	-	-	-	-	day after	NR
	#nbv^{3}	21/07/10	0.80	314°	0.76	321°	0.67	321°	day after	A

Table 1 Control Pigeons - RS: release site, home direction and distance; Track: track code and individual pigeon code number are reported; Date: date of release; r and α: mean vector length and directions relative to the sections of tracks recorded in the first (1), the two first (2) and the three first (3) hours after release (see Material and methods for further explanations); ht: homing time expressed in hours and minutes; “day after”, the pigeon homed the day after the release, “later”, the bird homed in the subsequent days, “lost” the bird never homed; at, analysis of the tracks: A, track used in the single release statistical analysis and P used also in the pooled analysis; I, incomplete track which could not be used in the statistical analysis; NR the track was not recorded by the logger. Release from Bolgheri: five out of 11 C pigeons tested at Bolgheri had previously tested at Montespertoli (two former C, two former PA and one former AO). Release from Montespertoli: Six out of 11 C pigeons tested at Montespertoli had been previously released at Bolgheri (3 former C and 3 former AO).

RS	Track	Date	r (1)	α (1)	r (2)	α (2)	r (3)	α (3)	ht	at
Bolgheri 336° 53 km	#ggbg^{18}	02/07/2010	0.44	334°	0.44	329°	0.46	331°	9^{h} 00’	A, P
	#ggr^{19}	02/07/2010	0.12	213°	0.08	307°	0.14	212°	Later	A, P
	#gm^{20}	02/07/2010	0.42	347°	0.52	337°	0.54	335°	4^{h} 50’	A, P
	#gr^{16}	02/07/2010	0.16	309°	0.15	311°	0.15	309°	Later	A, P
	#grgr^{21}	02/07/2010	0.64	322°	0.60	353°	0.13	305°	Later	A, P
	#gb^{22}	05/07/2010	0.66	116°	0.12	316°	0.11	314°	Later	A, P
	#gv^{23}	05/07/2010	0.53	331°	0.56	330°	0.22	332°	day after	A, P
	#gc^{24}	05/07/2010	0.78	328°	0.74	335°	0.72	335°	day after	A, P
Montespertoli 270° 61 km	#vv^{25}	07/07/2010	0.48	278°	0.47	277°	0.47	277°	day after	A, P
	#vrgr^{5}	07/07/2010	0.14	136°	0.18	236°	0.18	236°	day after	A, P
	#vr^{26}	07/07/2010	0.78	266°	0.77	266°	0.77	266°	8^{h} 35’	A, P
	#vm^{20}	07/07/2010	0.52	317°	0.38	301°	0.31	301°	7^{h} 05’	A
	#vgr^{11}	07/07/2010	0.74	312°	0.64	310°	0.64	310°	day after	A, P
	#vc^{10}	07/07/2010	0.16	280°	0.37	348°	0.04	347°	day after	A, P
	#vbcb^{27}	07/07/2010	0.27	332°	0.51	330°	0.66	303°	Lost	A, P

Table 2 Pure Air Pigeons. Explanations as in Table 1. Two out of 7 PA pigeons tested at Montespertoli had been released at Bolgheri (1 former C and 1 former PA).

RS	Track	Date	r (1)	α (1)	r (2)	α (2)	r (3)	α (3)	ht	at
Bolgheri 336° 53 km	#rgrg^{28}	02/07/2010	0.21	275°	0.38	357°	0.38	357°	day after	A, P
	#rrnr^{29}	02/07/2010	0.07	275°	0.07	275°	0.07	275°	later	A, P
	#rrbr^{13}	02/07/2010	0.20	324°	0.15	337°	0.15	337°	day after	A, P
	#rgng^{17}	02/07/2010	0.27	316°	0.31	319°	0.37	321°	day after	A, P
	#rgcg^{30}	02/07/2010	0.59	070°	0.58	073°	0.29	115°	lost	A, P
	#rc^{31}	05/07/2010	0.26	193°	0.14	190°	0.14	190°	later	A, P
	#rb^{32}	05/07/2010	0.06	191°	0.27	332°	0.26	334°	day after	A, P
	#rrnr2^{33}	05/07/2010	0.04	113°	0.22	236°	0.38	326°	day after	A, P
Montespertoli 270° 61 km	#mv^{34}	07/07/2010	0.34	302°	0.48	323°	0.43	299°	lost	A, P
	#mgr^{19}	07/07/2010	0.25	351°	0.06	046°	0.26	217°	day after	A
	#mgbg^{4}	07/07/2010	0.75	340°	0.48	284°	0.48	284°	5^{h} 00’	A, P
	#mc^{2}	07/07/2010	0.44	352°	0.07	328°	0.59	336°	later	A, P
	#mbcb^{35}	07/07/2010	0.34	242°	0.26	249°	0.26	249°	day after	A, P
	#mb^{7}	07/07/2010	0.03	162°	0.13	306°	0.12	307°	later	A, P
	#mr^{36}	07/07/2010	0.23	301°	0.45	333°	0.42	333°	lost	A, P

Table 3 Artificial Odour Pigeons - Explanations as in Table 1. Three out of 7 AO pigeons tested at Montespertoli had been previously used at Bolgheri (2 former C and 1 former PA).

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2.3 ORIENTATION OF GPS TRACKS WITH RESPECT TO THE MAGNETIC SLOPE: A COMPARISON BETWEEN INTACT AND TRIGEMINAL SECTIONED PIGEONS

ABSTRACT

Conditioning studies have shown that the ophthalmic branch of the trigeminal nerve, which is thought to innervate magnetite particles in the bird's upper beak, is functionally involved in the perception of the intensity of the geomagnetic field. However, homing experiments have demonstrated that trigeminal mediation of magnetoreception is neither necessary nor sufficient for pigeon navigation from unfamiliar distant locations. Nevertheless, some authors claim that pigeons' orientation at the release site is dominated by their tendency to fly either parallel or perpendicular to the magnetic isolines. Thus, a scattering of pigeons released over magnetic anomalies has been observed, which is reduced by anaesthesia of the upper beak. Furthermore, it has been suggested that pigeons' whole flight paths are shaped by geomagnetic intensity variations. To test the hypothesis that pigeons' flight paths are affected by the pattern of magnetic isolines, we have analysed GPS-tracks of two groups of pigeons: intact birds and birds with section of the ophthalmic branch of the trigeminal nerve. We have undertaken a statistical analysis of the deviation of the tracks from both the direction of the local geomagnetic gradient and the home direction at both the release sites and "en route". If the local geomagnetic intensity affects the orientation of birds, a difference between trigeminal sectioned and intact pigeons in both the initial orientation and the orientation of their whole flight path should be detectable. As concerning the initial orientation of the pigeons, we report that only the trigeminal sectioned pigeons seemed to be significantly oriented towards the magnetic steepest slope (but not towards the magnetic isolines). As concerning the homing flight paths analysis, no difference between the trigeminal sectioned pigeons and control pigeons was found. The tendency of both intact and trigeminal sectioned pigeons to orient parallel to the magnetic isolines, or alternatively to the magnetic slope, seemed to be determined by how much the home direction coincided with the direction of either the magnetic slope or the magnetic contours in the sampled fixes.

INTRODUCTION

One of the most debated questions in bird navigation is the nature of the cues used for a position finding mechanism - the so called “navigational map” of Kramer’s “map and compass” concept (Kramer, 1953). Forty years of research on homing pigeons have shown that, while a long lasting olfactory deprivation consistently produces a homing impairment of pigeons released from unfamiliar locations (Papi et al., 1972; Wallraff, 2005; Gagliardo et al., 2009b), no magnetic manipulation is able to consistently affect their homing success, although variable effects on initial orientation following application of either oscillating magnetic fields or magnetic pulses have been reported (Walcott et al., 1988; Beason et al., 1997; Wallraff, 1980; Wiltschko and Wiltschko, 1978; Papi et al., 1983). Conditioning experiments have suggested that pigeons perceive the changes in magnetic intensity through magnetite formations located in the upper beak and innervated by the ophthalmic branch of the trigeminal nerve (Williams and Wild, 2001; Fleissner et al., 2003b; 2003a). These results strengthened the idea that birds might use these upper beaks magnetic formations to gain geomagnetic information useful for a position finding mechanism (see (Cadiou and McNaughton, 2010) for a review). However, no navigational impairment following trigeminal section (Gagliardo et al., 2006; 2008a; 2009a) or extirpation of the lagena has been observed (Wallraff, 1972), questioning the involvement in navigation of the putative magnetoreceptive systems proposed so far, i.e., the magnetite particles innervated by the ophthalmic branch of the trigeminal nerve in the beak (Williams and Wild, 2001; Fleissner et al., 2003b; 2003a) and the lagena (Wu and Dickman, 2011).

Despite the fact that nerve section experiments contradict the hypothesis that pigeons rely on geomagnetic intensity for homing (Walker, 1998; 1999) (see also (Wallraff, 1999)), it has been reported that pigeons at the release site tend to orient parallel either to the steepest magnetic slope or to the magnetic isolines (Mora and Walker, 2009; Dennis et al., 2007). This tendency would also produce systematic and predictable errors in homeward orientation depending on the direction of the local magnetic gradient (Walker, 1998; 1999). It has been recently reported in a GPS study that pigeons released in magnetic anomaly take longer time to orient homeward and have more difficulties in determining the home direction than pigeons released in magnetically quite terrain (Schiffner et al., 2011). The involvement of the putative trigeminal receptor in the upper beak in disorientation occurring at magnetic anomalies release sites has been suggested, as pigeons subjected to local anaesthesia of the upper beak displayed vanishing bearing distribution better homeward oriented than un-manipulated birds

(Wiltschko et al., 2010). The authors claimed that pigeons normally rely on trigeminal receptor mediated geomagnetic information for establishing their position with respect to home and therefore are confused by the magnetic anomaly; rendered unable to sense the local geomagnetic intensity, the pigeons use other navigational cues to orient homeward and home. Therefore, according to this view, the putative magnetoreceptor in the upper beak, although not necessary for navigation, would in some way be involved in the navigational process within a multi-factorial system.

It is well known that pigeons display a homeward orientation already a few minutes after release (Wallraff, 2005) and even before takeoff (Chelazzi and Pardi, 1972; Mazzotto et al., 1999). This implies that they use the map mechanism soon after release in order to decide in which direction to fly. Although the use of geomagnetic intensity information is supposedly used at the release site, it has been recently suggested that geomagnetic field parameters affect the whole flight path of pigeons, well beyond their decision point (Schiffner and Wiltschko, 2011).

In order to test the role of geomagnetic field intensity in initial orientation at the release site and in shaping the flight path during the homeward journey, we compared tracks of both trigeminal sectioned pigeons and intact/sham operated birds, specifically by measuring the birds’ orientation with respect to the direction of the steepest magnetic field intensity gradient. If magnetic field intensity is a critical component of the navigational map we should be able to observe a difference of intact and trigeminal sectioned pigeons in response to the direction of the steepest magnetic slope at the release site. In particular, we would expect the intact birds to tend to orient either perpendicular or parallel to the direction of the steepest magnetic slope soon after release (Mora and Walker, 2009; Dennis et al., 2007). If the pattern of geomagnetic field intensity affects navigational decisions en route, even if pigeons do not exclusively rely on this kind of information in a position finding mechanism (Schiffner and Wiltschko, 2011), we should be able to observe a systematic tendency of the birds to orient either perpendicular or parallel to the magnetic isolines in the intact pigeons, but not in the trigeminal sectioned group.

MATERIALS AND METHODS

All the birds used in the experiments were adult homing pigeons that had been bred and hatched at the Arnino field station near Pisa (latitude 43°39’26’’N, longitude 10°18’14’’ E). The birds were kept according to the Italian laws on animal welfare and were allowed to perform spontaneous flights around the loft. Before being released the birds were equipped with GPS data loggers. The birds subjected to section of the ophthalmic branch of the trigeminal nerve (Tr) had been used previously in experiments reported in previous papers, where the surgical procedure is described (Gagliardo et al., 2006; 2009b). Birds receiving trigeminal section were examined post mortem under a high-powered surgical microscope for signs of nerve re-growth. GPS tracks from those birds in which there was such evidence, which consisted of highly abnormal sprouting from the proximal stump on one or both sides, were discarded from the analysis. The control birds (C) were either unmanipulated or subjected to sham surgery (see below).

Experiment 1 Orientation at the release site area. The analysis of the initial orientation of the birds with respect to the Earth’s magnetic field was performed on tracks from various sources: (a) tracks of unmanipulated pigeons published in previous papers [28,29,30], (b) on the tracks of sham operated control and sectioned pigeons reported in Experiment 2, (c) on additional unpublished tracks of intact birds released from Bolgheri. All the birds were unfamiliar with the release sites. The tracks considered in the analysis were homing routes of birds released at the following sites: Marinella, latitude 44° 03’ 38’’ N, longitude 09° 59’ 49’’ E; home direction 154°, distance 57.4 km (8 Tr and 5 C birds); Bolgheri, latitude 43° 13’ 04’’ N, longitude 10° 34’ 18’’ E; home direction 336°, distance 54.8 km (5 Tr and 17 C); Empoli, latitude 43° 42’ 46’’ N, longitude 10° 55’ 08’’ E; home direction 262°, distance 50.0 km (7 Tr and 7 C); Massaciuccoli, latitude 43° 49’ 09’’ N, longitude 10° 20’ 49’’ E; home direction 186°, distance 19,7 km (13 C) ; Cigoli, latitude 43° 33’ 38’’ N, longitude 10° 39’ 48’’E; home direction 267°, distance 41,6 km (7 C); Fucecchio, latitude 43° 42’ 26’’ N, longitude 10° 43’ 16’’ E; home direction 260°, distance 34.0 km (7 C).

Experiment 2 Orientation along route. All the pigeons had been previously used in other experiments (Gagliardo et al., 2006; 2008; 2009) in which, after a surgical treatment, each bird was released only once, either from south or north with respect to home. In particular, all the birds released from Bolgheri had been previously tested from Marinella, and all the birds released from Marinella had been previously tested from Bolgheri. All the birds released at Empoli had a homing experience from both Marinella and Bolgheri. The surgical treatment consisted of a bilateral section of the ophthalmic branch of the trigeminal nerve or sham surgery (see for details on the surgery (Gagliardo et al., 2006; 2008; 2009).

Before being released singly, the pigeons were equipped with a GPS data logger (www.technosmart.it) in order to record their flight path. All the experimental releases took place in sunny conditions, with no or light wind. The experiments were conducted from three release sites: Empoli, Bolgheri and Marinella located East, South and North-West of home, respectively.

Analysis of the tracks. The miniature GPS data loggers allow the recording of the flight path with an accuracy of about 4 m (Steiner et al., 2000; Lipp et al., 2004). Every second the GPS data loggers recorded latitude, longitude, speed, and time of recording and altitude. Altitude data were not reliable and therefore not considered in the analysis.

The individual tracks were visualised with MAPINFO software (One Global View, Troy, NY 12180) and drawn on a map of the magnetic anomaly of Italy (Chiappini et al., 2000), scanned and loaded in MAPINFO. The tracks analysed in this paper can be inspected in Movebank (www.movebank.org).

The area under investigation was converted into a regular grid from longitude 9.58° to 11.18° E at steps of 0.04° (about 3 km) and from latitude 42.76° to 44.26° N at steps of 0.03° (about 3.3 km). Then, the direction of the magnetic gradient (θ) was calculated at each intersection of this grid (Fig. 3) from the observed magnetic field. It is known that the magnetic field gradient is determined by the rate of change of the strength of the field over distance. A greater change in a fixed distance corresponds to a steeper slope. Here the geomagnetic field gradient was estimated taking into account that the typical magnetic declination is less than 2° in the investigated area (Maus et al., 2000), so the gradient can be simply estimated on the basis of the geomagnetic anomaly field (Chiappini et al., 2000). Taking the gradients for each x (East) and y (North) direction as f_x and f_y, the total gradient f and the clockwise direction δ (with respect to North) of the magnetic slope are given by:

$$f = \sqrt{f_x + f_y}\ ; \qquad \delta = \operatorname{atan}\left(\frac{f_x}{f_y}\right) \text{, respectively.}$$

The smallest angular distance between the home direction and θ, or θ+180, or θ+/-90° for each release site is as follows: Marinella 14°, Massaciuccoli 13°, Fucecchio 40°, Cigoli 36°, Bolgheri 10°, Empoli 33°.

For the analysis we have considered only the active flight of the birds by excluding all the fixes recorded when the speed was lower than or equal to 5 km per hour. For each track we calculated the moving average of ten consecutive latitude and longitude values, in order to reduce the noise of the flight itself and to obtain the main orientation kept by the birds in about 10 seconds. For the analysis of the initial orientation (Experiment 1) we considered the portion of the tracks between 1 and 3 km from the release site. Afterwards, we considered the orientation of each bird while moving from one average fix to the next, and we calculated the mean direction and its deviation from the direction of both home and the magnetic slope. In addition we performed the Hotelling test on the so calculated mean vectors to test for randomness both C and Tr birds' initial orientation distribution. In the analysis of the whole tracks (Experiment 2), we considered the portions of the tracks less than 1.5 km distant from each intersection of the grid, providing that they were more than 1 km distant from the release site and more than 10 km from home. The latter criterion aimed at excluding from the analysis an area which was likely to be familiar to the birds. Afterwards, we considered the orientation of the birds while moving from one average fix to the next and calculated its deviation from both the home direction and the local magnetic slope direction. For both Experiment 1 and 2 the deviations in absolute values were grouped, as in the analysis performed by Mora and Walker, in 6 categories (see Figure 2 in Mora and Walker, 2009) ranging as follows: category 1, 0°-15° or 166°-180°; category 2, 16°-30° or 151°-165°; category 3, 31°-45° or 136°-150°; category 4, 46°-60° or 121°-135°; category 5, 61°-75° or 106°-120°; category 6, 76°-90° or 91°-105°. According to the hypothesis that pigeons orient by following either the geomagnetic gradient or isolines, the frequency distribution of the deviations should be U shaped, i.e., most of the deviations should fall in categories 1 and 2 and 5 and 6. According to the expected frequency distribution expressing the best orientation towards the home direction, the frequency of deviations should be greater in categories 1 and 2, while if the birds oriented preferentially parallel to the magnetic isolines most of the deviations should fall in categories 5 and 6. The observed frequency distributions of deviations were compared to the expected distributions by means of the Spearman correlation test (Spearman, 1904).

The procedure is as follows. We considered the distribution of the observed deviations from the direction of the magnetic gradient in six categories, according to Mora and Walker (2009). We then attributed a rank to each category, with the highest rank given to the category including the greatest number of samples. When we tested a U shaped expected distribution (according to Mora and Walker (2009)), the ranks will be as follows: category 1 and 6, rank 5.5; category 2 and 5, rank 3.5; category 3 and 4, rank 1.5. When we tested if the birds preferentially maintain the magnetic gradient direction or the home direction the ranks will be as follows: categories 1 and 2, ranks 5.5, categories 3, 4,5,6, ranks 2.5. The following formula is used to calculate the Spearman correlation coefficient *r* (Spearman, 1904) between the observed and the expected frequency distributions:

$$r = 1 - \frac{6\sum_{i} A_i - B_i{}^{2}}{n(n^2-1)}$$

where A_i is the rank of each category from *i* to *n* for the observed distribution and B_i the rank of each category from *i* to *n* for the expected distribution; *n* is the number of categories. In the case of the analysis relative to the deviations from the direction of the magnetic gradient, we assumed the ranks for the categories of the expected distribution as follows: category 1, rank 6; category 2, rank 5, category 3, rank 4; category 4, rank 3; category 5, rank 2; category 6, rank 1. In the case of the analysis relative to the deviation from the magnetic isolines we assumed the ranks for the categories of the expected distribution as follows: category 1, rank 1; category 2, rank 2, category 3, rank 3; category 4, rank 4; category 5, rank 5; category 6, rank 6. In the case of the analysis relative to the deviation from the home direction we assumed the ranks for the expected distribution as follows: category 1 and 2, rank 5.5; category 2, 3, 4, 5 and 6, rank 2.5.

In Experiment 1 the Spearman correlation test was applied to the deviations from the magnetic gradient direction and from the home direction displayed in the release site area.

In Experiment 2 the Spearman correlation test was applied to the deviations from the geomagnetic gradient and isolines direction and from the home direction displayed by each single bird along the route. In order to determine whether the coincidence between the home direction and the direction of either the magnetic gradient or the magnetic isolines increased the probability that the birds significantly oriented along these directions, we correlated the percentage of sampled fixes in which the home direction deviated less than or equal to 30° from the direction of either the magnetic gradient or the magnetic isolines with the percentage of deviations falling in both category 1 (0-15°) and 2 (16-30°).

RESULTS

Experiment 1. Orientation at the release site area

The sections of the tracks in the vicinity of the release site are shown in Fig. 1 for both intact and trigeminal sectioned pigeons (see Table 1 for details). Both groups’ mean vector distributions calculated on the section of the track in the release site area turned out to be significantly different from random (second order mean vectors C: $r=0.41$ $\alpha=001°$ Hotelling test $p<0.001$; Tr $r=0.28$ $\alpha=355°$, $p<0.05$). The frequency distribution of the deviations from the home direction are significantly correlated to the expected distribution predicting an homeward orientation in both groups (Spearman test, C $n= 56$ $r = 0.842$, $p<0.05$; Tr $n= 20$ $r = 0.900$, $p<0.025$). The Spearman correlation test showed that the deviations (see Figure 2) from the direction of the magnetic gradient are not significantly correlated with the expected U-shaped frequency distribution for either intact or trigeminal sectioned pigeons (Spearman test, C $r = -0.300$; Tr, $r = 0.085$). While the intact pigeons do not display a tendency to orient parallel to the magnetic slope direction (Spearman test, C $r = 0.500$), the trigeminal sectioned birds turned out to significantly prefer the magnetic gradient direction (Tr $r = 0.885$, $p<0.05$). According to the Spearman test both experimental groups did not show a preferential orientation towards the magnetic isolines direction (C $r= -0.528$, Tr $r= -0.428$).

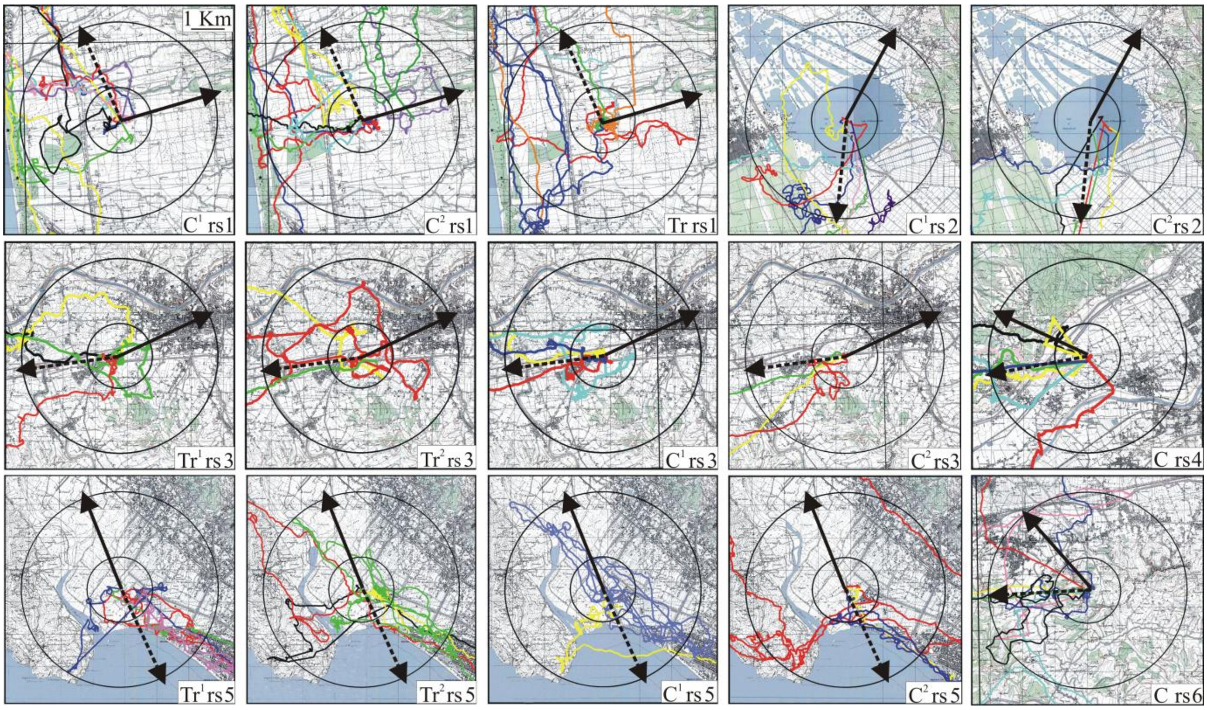

Fig. 1 Experiment 1. Each square reports the initial orientation of the tracks; the superscript numbers indicate the same set of data for which the tracks are represented separately. Each coloured line represents the track of one pigeon. The portion of the tracks included in the analysis is delimited by the two circles of 1 and 3 km radius, respectively. C and Tr indicate the treatments, control and trigeminal sectioned pigeons, respectively. Release sites (rs) numbered from 1 to 6 are in the order as follows: Bolgheri, Massaciuccoli, Empoli, Fucecchio, Marinella, Cigoli. The broken arrow and the continuous arrow represent the home direction (h) and the magnetic upward gradient direction (θ), respectively. The magnetic gradient direction used in the analysis is that relative to the intersection of the grid closest to the release site.

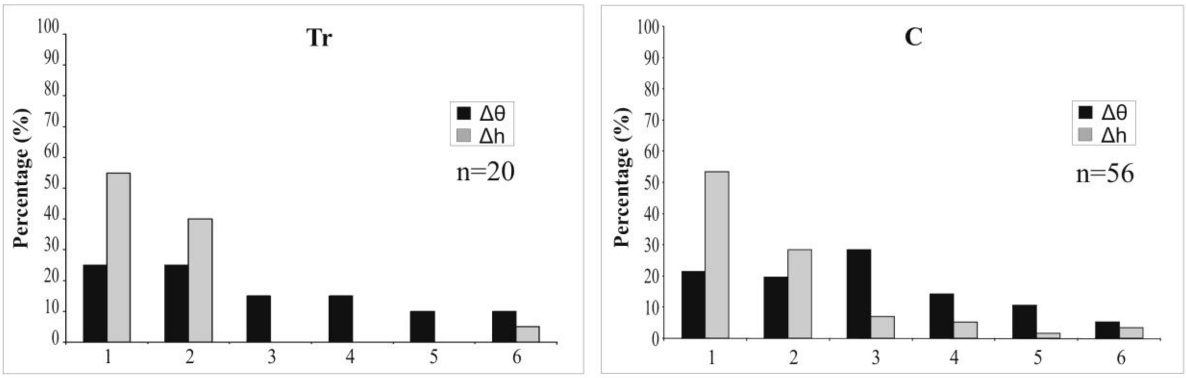

Fig. 2. Experiment 1. Frequency distribution of the deviations from both the magnetic gradient (Δθ) and the home (Δh) directions recorded in the vicinity of the release site. The six categories indicated in the diagram include the deviations ranging between the angles as follows. 1: 0°-15° or 166°-180°; 2: 16°-30° or 151°-165°; 3: 31°-45° or 136°-150°; 4: 46°-60° or 121°-135°; 5: 61°-75° or 106°-120°; 6: 76°-90° or 91°-105°. See Materials and methods for further explanations.

Experiment 2. Orientation along route

The tracks of both C and Tr from Bolgheri, Marinella and Empoli plotted over the magnetic anomaly map are reported in Fig. 3. The frequency distribution of the deviations from the direction of the magnetic gradient is never significantly correlated with the expected U-shaped frequency distribution (Spearman test, see Table 2 for details).

However, when we assumed an expected frequency distribution predicting a preferential orientation towards the magnetic slope direction the Spearman correlation test gave significant results for 3 out of 9 C and 4 out of 13 Tr birds in the test from East (Empoli), and 1 out of 6 C and 8 out of 11 Tr pigeons released from North-West (Marinella). A significant tendency to fly parallel to the magnetic isolines was highlighted only at Bolgheri and for all released pigeons belonging to both experimental groups. According to the χ^2 test the two experimental groups behaved similarly in their tendency to follow either the magnetic gradient or the magnetic isolines (C vs Tr, $p>0.05$ in all comparisons) (see Table 3 for details). Considering the deviations of the birds from the home direction at the sampled fixes, the frequency distribution was not significantly correlated to the expected distribution in a minority of cases: 1 out of 13 Tr birds released from Empoli, 4 out of 13 C and 2 Tr out of 6 birds released from Bolgheri, and 3 out of 6 C pigeons and 1 Tr out of 11 pigeons from Marinella (χ^2 test C vs Tr, $p>0.05$ in all comparisons) (Table 2).

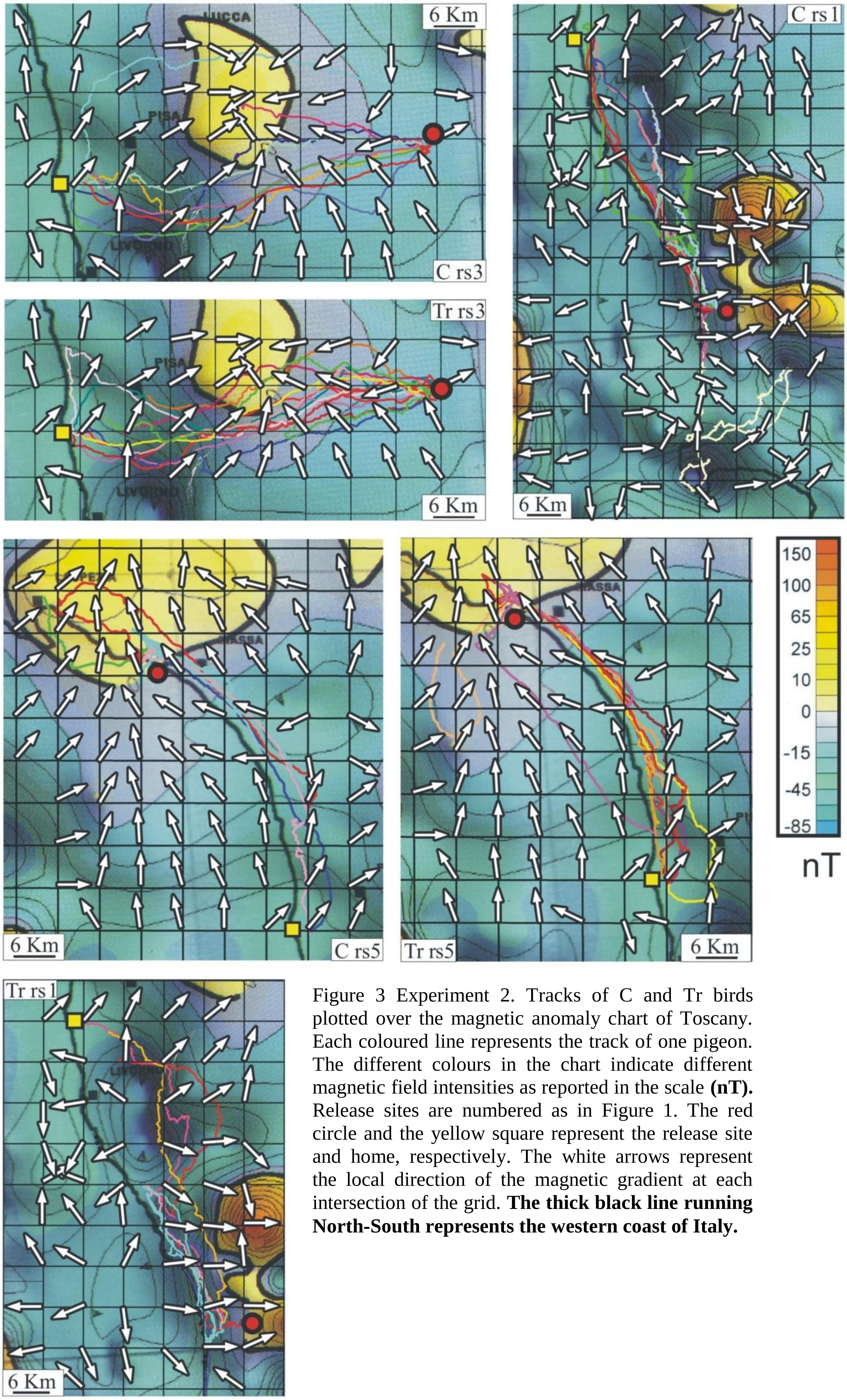

Figure 3 Experiment 2. Tracks of C and Tr birds plotted over the magnetic anomaly chart of Toscany. Each coloured line represents the track of one pigeon. The different colours in the chart indicate different magnetic field intensities as reported in the scale **(nT).** Release sites are numbered as in Figure 1. The red circle and the yellow square represent the release site and home, respectively. The white arrows represent the local direction of the magnetic gradient at each intersection of the grid. **The thick black line running North-South represents the western coast of Italy.**

The coincidence (+/- 15°) between the home direction and the direction of either the magnetic isolines or the magnetic gradient seemed to affect the results of the Spearman correlation test. In fact (see Fig. 4) the percentage of deviations falling in categories 1 (0-15°) and 2 (16-30°) is significantly correlated with the percentage of fixes at which the direction of the magnetic gradient (Linear correlation r= 0.649, p<0.001) or the direction of the magnetic isolines (Linear correlation r= 0.892, p<0.001) are roughly parallel (maximal deviation 30°) with the home direction.

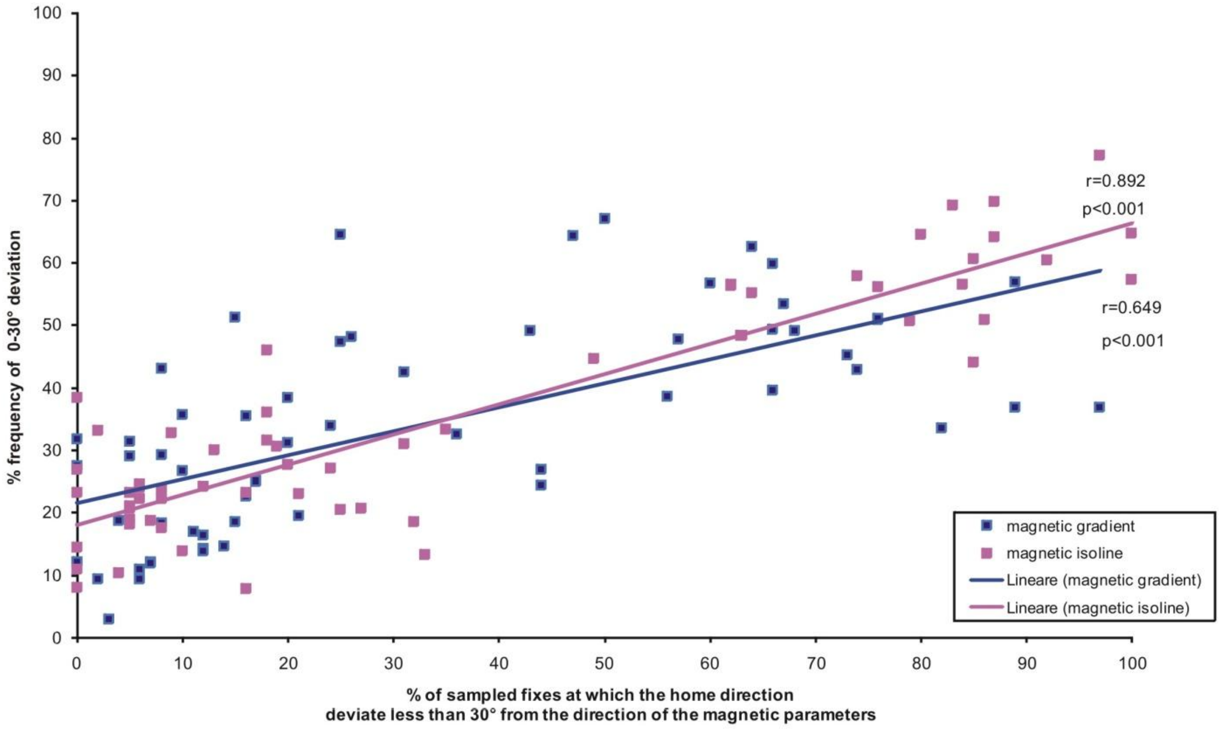

Fig. 4. Experiment 2. Correlation between the percentage of sampled fixes (n%) in which the home direction is coincident (+/- 30°) with the direction of either the magnetic gradient or the magnetic isolines and the percentage of deviations falling in categories 1 and 2. r: linear correlation coefficient. The number of tracks considered is 59.

DISCUSSION

Our GPS data confirm what has been observed with tests performed with traditional methods, that is, trigeminal sectioned pigeons are unimpaired in homing (Gagliardo et al., 2006; 2008; 2009). However, the main aim of the present work was to provide a detailed analysis of the birds' orientation with respect to the direction of the magnetic gradient, either restricted to the release site area or along the whole route.

The analysis of the initial orientation of the birds failed to highlight a pattern similar to that reported by Mora and Walker on the basis of the results of a meta-analysis (Mora and Walker, 2009). These authors observed that either first- or second-order mean vector directions, derived from numerous experiments conducted at different release sites in Germany, are mostly oriented either parallel or perpendicular to the magnetic slope. From this observation they concluded that pigeons have the tendency to fly either parallel or perpendicular to the magnetic slope. It should be noted, however, that the result shown by Mora and Walker can be achieved even if no single bird flew parallel or perpendicular to the isolines, as both a first-order and a second-order mean vector direction can originate from a distribution in which none of the pigeons orient either parallel or perpendicular to the magnetic slope. A further problem with the analysis of Mora and Walker (2009) is their use of vanishing bearings. These do not indicate the actual direction of the bird's movement but simply indicate the bird's direction with respect to the release site at a certain distance from it (typically when the bird vanishes from the observer's view). Clearly, it is critically important to assess the bird's movement in order to determine whether the bird is flying along a magnetic isoline or along a magnetic gradient.

However our analysis has shown that the trigeminal sectioned pigeons, but not the control pigeons, significantly preferred to orient parallel to the direction of the magnetic steepest slope in the release site area. This finding is in contrast to what observed in previous studies (Wiltschko et al., 2010), that initial orientation of pigeons subjected to anaesthesia of the upper beak trigeminal nerve endings was not affected by the presence of a magnetic anomaly at the release site, while the control pigeons turned out to be scattered.

As trigeminal nerve section does not impair homing ability of homing pigeons (Gagliardo et al., 2006, 2008a, 2009a), and present paper], it is clear that the putative magnetic receptor in the upper beak is not involved in the navigational map mechanism purportedly used to home from unfamiliar sites. However, the perception of the magnetic field intensity might affect the directional choices of the birds (Wiltschko et al., 2010; Schiffner and Wiltschko, 2011) either at the release site and/or “en route”, similarly to what happens for the visual features of over-flown areas (Lipp et al., 2004; Lau et al., 2006; Bonadonna et al., 1997). In this case we would expect a different behaviour between the control and trigeminal sectioned pigeons. According to our data, pigeons seem not to be affected by the local magnetic landscape. In this case we would expect a different behaviour between the control and trigeminal sectioned pigeons. By contrast both groups displayed the same behaviour in relation to the magnetic isoline pattern of the over-flown areas. In fact, we could not observe any difference in the directional choices of birds perceiving or not perceiving magnetic intensity information through the putative trigeminal magnetoreceptor.

From the analysis of the whole tracks it emerged that, “en route”, all the pigeons belonging to both groups released from Bolgheri flew orienting significantly parallel to the magnetic isolines. This tendency was never observed at Marinella and Empoli. In fact from these sites the birds (especially the trigeminal sectioned pigeons) tended to orient parallel to the magnetic slope direction. The birds heading home from Bolgheri cross a wide area in which the magnetic isolines direction coincides with the home direction, while the birds heading home from either Marinella and Empoli fly over terrain in which the magnetic slope direction is coincident with the home direction. This implies that birds heading home with a non magnetic mechanism have a high probability to orient towards the magnetic isolines or toward the magnetic steepest slope. If the magnetic contours would directly and specifically affect the directional choices of the birds we should have observed a number of pigeons oriented along the magnetic isolines also at Empoli and Marinella. In the same way, is the birds would be specifically affected by the direction of the steepest magnetic slope, birds oriented parallel to the direction of the magnetic gradient should have been observed also at Bolgheri. In addition, occasional alignment of the tracks parallel or perpendicular to the magnetic slope is likely to be produced by chance when local topographic features, as the coastline or valleys and hills, actually shape the birds’ local orientation.

In conclusion we were unable to observe an effect of the local magnetic intensity on the orientation of the intact control pigeons in the initial phase of the homing journey, that is, in the vicinity of the release site where, according to the map and compass concept (Kramer, 1953), the birds are able to establish their new position with respect to the goal and orient towards it. It is worth noting that the trigeminal sectioned birds’ orientation seemed to be influence by the direction of the magnetic gradient and that for these birds the detection of magnetic field intensity was presumably unavailable.

Release site biases in birds orientation have been often attributed to irregularities of the magnetic field intensity variation, and therefore directly caused by local magnetic anomalies (Walker, 1998). In our case the trigeminal sectioned birds' orientation considered in the initial orientation analysis turned out to preferentially orient towards the direction of the magnetic slope. However these birds were also homeward oriented, as well as the intact controls pigeons. Looking at the behaviour of the pigeons along route, it emerged that both for Marinella and Empoli data set, the birds displaying some uncertainty in their homeward orientation (see Table 2) did not seem to be influenced by the geomagnetic field irregularities. At Bolgheri, both birds maintaining a homeward orientation during the whole route and the birds heading away from home flying south along the coast in some parts of their journey, significantly oriented parallel to the magnetic contours. As reported in the literature (Wallraff 2005), the pigeon orientation is often affected by previous homing experience and this might explain the orientation toward south of some birds tested at Bolgheri, South from home, that had their first homing experience from Marinella, North from home. Similarly some birds released at Marinella (and previously release at Bolgheri) followed the coast in a direction opposite from home, as if they were at Bolgheri.

In conclusion, we have been unable to observe a consistent and specific effect of the geomagnetic field variation on the orientation of the intact control pigeons, which was not present in the supposedly magnetically deprived pigeons and which was not correlated to the coincidence between the home direction and the direction of either the magnetic slope or the direction of the magnetic isolines.

TABLES

RS	T	Track	α	Δm	Δh	RS	T	Track	α	Δm	Δh
Marinella 154° 57.4 Km; θ 320°	C	GGrGc1	147°	173°	-5°	Bolgheri 336° 54.8 Km; θ 076°	C	GBGc2	297°	-139°	-39°
		GCFc1	139°	-179	-13°			GGrGc2	288°	-148°	-48°
		362c	265°	-55°	113°			RMRc2	300°	-136°	-36°
		BCBc	167°	-153°	15°			Blib	209°	133°	-127°
		Vc	134°	174°	-18°			Glib	142°	66°	-166°
	Tr	BGBtr1	126°	166°	-26°			RVRlib	329°	-107°	-7°
		CGrCt1	123°	163°	-29°			NVNlv	299°	-137°	-37°
		CNCt1	211°	-109°	59°			VGrVl	272°	-164°	-64°
		CRCtr1	116°	156°	-36°			VNVlv	310°	-126°	-26°
		GNGtr1	175°	-145°	23°			NGNlv	329°	-107°	-7°
		RBRtr1	123°	163°	-29°			VCVlv	25°	-51°	49°
		RCRtr1	151°	-169°	-1°			269c	168°	92°	-168°
		RGrRtr1	166°	-154°	14°			Grc	60°	-16°	-84°
Massaciuccoli 186° 19.7 Km; θ 019°	C	gcglib	174°	155°	-12°			Mc	311°	-125°	-25°
		grg1li	173°	154°	-13°			NGNc	287°	-147°	-49°
		grg1lib	175°	156°	-11°			RVRc	283°	-153°	-53°
		rcrlib	182°	163°	-4°			VNVc	284°	-152°	-52°
		rbrlib	185°	166°	-1°		Tr	BCBtr2	304°	-132°	-32°
		bcon	190°	171°	4°			BMBtr2	313°	-123°	-23°
		cbccon	276°	-103°	90°			BNBtr2	162°	86°	-174°
		ccong	194°	175°	8°			CMCtr2	332°	-104°	-4°
		g-g	236°	-143°	50°			CNCtr2	312°	-124°	-24°
		rcong	284°	-95°	98°	Empoli 262° 50 Km; θ 295°	C	GBGc1	264°	-31°	2°
		766lv	156°	137°	-30°			GRGc1	303°	8°	41°
		856lv	202°	177°	16°			Nc1	254°	-41°	-8°
		867lv	188°	169°	2°			RMRc2	275°	-20°	13°
Fucecchio 260° 34 Km; θ 300°	C	c180	293°	-7°	33°			Cc	261°	-34°	-1°
		c513	278°	-22°	18°			GNGc	231°	-64°	-31°
		c595	266°	-34°	6°			RBRc	223°	-72°	-39°
		c743	258°	-42°	-2°		Tr	BGrBtr2	221°	-74°	-41°
		c056	233°	-67°	-27°			NBNtr2	309°	14°	47°
		c 150	301°	1°	41°			BVBtr2	252°	-43°	-10°
		c 170	263°	-37°	3°			CBCtr2	305°	10°	43°
Cigoli 267° 41.6 Km;θ 303°	C	c181	258°	-45°	-9°			RGRtr1	308°	13°	46°
		c551	269°	-34°	2°			RNRtr1	313°	18°	51°
		c757	183°	-120°	-84°			GGrGtr	260°	-35°	-2°
		c787	343°	40°	76°						
		c798	304°	1°	37°						
		c812	267°	-36°	0°						
		c872	254°	-49°	-13°						

Table 1. Initial orientation of the tracks. RS: Release site parameters; home direction, distance from home and direction of the magnetic gradient (θ). T: treatments; C, intact control pigeons, Tr, pigeons subjected to section of the ophthalmic branch of the trigeminal nerve. Track: individual pigeon code; the tracks can be inspected in Movebank (www.movebank.org). α: mean direction of the track recorded between 1 and 3 km from release. Δm: deviation from the direction of the magnetic gradient. Δh deviation from the home direction.

RS	**C**				**Tr**			
	Track	**r (θ)**	**r (h)**	**n**	**Track**	**r (θ)**	**r (h)**	**n**
Empoli	GBGc1	0.042	0.842*	1198	BGBtr1	0.271	0.842*	745
	GGrGc1	-0.642	0.842*	484	BGrBtr2	0.614	0.842*	357
	GRGc1	0.157	0.842*	246	NBNtr2	-0.757	0.842*	842
	Nc1	-0.871	0.842*	691	BVBtr2	0.271	0.842*	599
	RMRc2	0.385	0.842*	835	CBCtr2	0.5	0.842*	866
	BCBc	-0.871	0.857*	1558	CMCtr2	-0.185	0.842*	624
	Cc	-0.757	0.900*	392	CNCtr1	-0.071	0.842*	774
	GNGc	-0.3	0.857*	2036	CRCtr2	0.157	0.857*	220
	RBRc	-0.871	0.985**	1386	GNGtr1	0.042	0.671	464
					RCRtr1	-0.871	0.842*	551
					RGRtr1	-0.628	0.842*	789
					RNRtr1	0	0.842*	697
					GGrGtr	-0.185	0.842*	79
Bolgheri	GBGc2	0.157	0.900*	290	BCBtr2	0.457	0.842*	1378
	GGrGc2	0.042	0.842*	465	BMBtr2	0.114	0.842*	608
	RMRc2	0.042	0.857*	881	BNBtr2	0.157	0.157	1362
	269c	0.728	-0.014	1825	CMCtr2	0.271	0.842*	423
	Cc	0.042	0.857*	2307	CNCtr2	0.157	0.671	1578
	Grc	0.042	0.671	4243	CRCtr2	0.042	0.842*	880
	Mc	0.042	0.985**	167				
	NGNc	0.042	0.842*	560				
	RBRc	0.042	0.842*	494				
	RVRc	0.157	0.671	1983				
	VNVc	0.042	0.842*	2884				
	Gc	0.042	0.671	2727				
	GNG	0.042	0.842*	1425				
Marinella	GGrGc1	0.385	0.500	1730	CGrCtr1	0.157	0.857*	496
	GCGc1	-0.185	0.842*	961	BGBtr1	0.042	0.842*	936
	BCBc	-0.071	0.857*	405	CNCtr1	-0.185	0.842*	615
	Vc	-0.3	0.842*	2418	CRCtr1	0.157	0.842*	713
	269c	0.614	-0.014	1647	GNGtr1	-0.871	0.842*	460
	RVRc	-0.414	0.671	2898	RBRtr1	0.157	0.857*	766
					RCRtr1	0.157	0.842*	933
					RGrRtr1	0.385	0.842*	1091
					GGrtr	0.042	0.857*	2002
					NGrNtr	-0.071	0.900*	674
					Mtr	0.742	-0.014	2580

Table 2. Spearman correlation test results on track orientation. RS: release site. Track: individual code of the track; the tracks can be inspected in Movebank (www.movebank.org). C: intact control pigeons; Tr: pigeon subjected to the section of the ophthalmic branch of the trigeminal nerve. r (θ): Spearman correlation coefficient relative to the frequency distribution of the deviations from the direction of both the magnetic gradient and magnetic isolines. r(h): Spearman correlation coefficient relative to the frequency distribution of the deviations from the home direction. *, **,*** indicate the significance levels of the test p<0.05, p<0.01, p<0.0025, respectively. n: number of sampled angles.

RS	C				Tr			
	Track	**r (θ)**	**r (ι)**	**n**	**Track**	**r (θ)**	**r (ι)**	**N**
Empoli	GBGc1	0.842*	-0.528	1198	BGBtr1	0.842*	-0.185	745
	GGrGc1	0.328	-0.528	484	BGrBtr2	0.500	0.500	357
	GRGc1	0.842*	-0.357	246	NBNtr2	-0.357	0.157	842
	Nc1	-0.357	-0.014	691	BVBtr2	0.842*	-0.185	599
	RMRc2	0.842*	-0.185	835	CBCtr2	0.157	0.328	866
	BCBc	-0.185	-0.185	1558	CMCtr2	-0.014	-0.357	624
	Cc	-0.085	-0.257	392	CNCtr1	0.671	-0.528	774
	GNGc	0.500	-0.528	2036	CRCtr2	0.842*	-0.357	220
	RBRc	-0.185	-0.185	1386	GNGtr1	-0.357	0.671	464
					RCRtr1	-0.014	-0.357	551
					RGRtr1	0.000	-0.342	789
					RNRtr1	0.857*	-0.514	697
					GGrGtr	-0.014	-0.014	79
Bolgheri	GBGc2	-0.357	0.842*	290	BCBtr2	-0.528	0.842*	1378
	GGrGc2	-0.528	0.842*	465	BMBtr2	-0.514	0.857*	608
	RMRc2	-0.528	0.842*	881	BNBtr2	-0.528	0.842*	1362
	269c	0.157	0.842*	1825	CMCtr2	-0.528	0.842*	423
	Cc	-0.528	0.842*	2307	CNCtr2	-0.528	0.842*	1578
	Grc	-0.528	0.842*	4243	CRCtr2	-0.528	0.842*	880
	Mc	-0.528	0.842*	167				
	NGNc	-0.528	0.842*	560				
	RBRc	-0.528	0.842*	494				
	RVRc	-0.528	0.842*	1983				
	VNVc	-0.528	0.842*	2884				
	Gc	-0.528	0.842*	2727				
	GNG	-0.528	0.842*	1425				
Marinella	GGrGc1	0.500	-0.657	1730	CGrCtr1	0.842*	-0.357	496
	GCGc1	0.671	-0.771	961	BGBtr1	0.842*	-0.528	936
	BCBc	0.842*	-0.528	405	CNCtr1	0.671	-0.357	615
	Vc	0.500	-0.528	2418	CRCtr1	0.842*	-0.357	713
	269c	0.1671	-0.157	1647	GNGtr1	-0.014	-0.357	460
	RVRc	0.500	-0.528	2898	RBRtr1	0.842*	-0.357	766
					RCRtr1	0.842*	-0.528	933
					RGrRtr1	0.842*	-0.357	1091
					GGrtr	0.842*	-0.528	2002
					NGrNtr	0.842*	-0.528	674
					Mtr	0.0428	0.257	2580

Table 3. Spearman correlation test results on track orientation. RS: release site. Track: individual code of the track; the tracks can be inspected in Movebank (www.movebank.org). C: intact control pigeons; Tr: pigeon subjected to the section of the ophthalmic branch of the trigeminal nerve. r(θ): Spearman correlation coefficient relative to the frequency distribution of the deviations from the direction of the magnetic gradient. r (ι) Spearman correlation coefficient relative to the frequency distribution of the deviations from the direction of the magnetic isolines. *, **,*** indicate the significance levels of the test $p<0.05$, $p<0.01$, $p<0.0025$, respectively. n: number of sampled angles.

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2.4 ROLE OF THE FAMILIAR RELEASE SITE FEATURES IN RE-ORIENTATION AFTER CLOCK-SHIFT : A STUDY WITH GPS DATA LOGGERS.

ABSTRACT

It is known that pigeons are able to memorise the landscape features of both the release site and the over-flown areas. These might be used by the birds in association with the home direction from those locations (*site specific compass orientation* strategy). Alternatively pigeons might use the spatial relationships among single landmarks to head home (*piloting* strategy), without relying on the compass mechanism. In order to highlight which release site specific factors may favour the preferential use of one strategy, we put in conflict the topographical information with the compass information by shifting the internal clock of homing pigeons released from three different familiar sites. The analysis of the GPS tracks suggested that the characteristic features of the release site affect the level of reorientation after clock shift. In particular, the vicinity of the sea seems to determine a preference for the piloting strategy and by consequence a more efficient re-orientation.

INTRODUCTION

As concerning familiar landmarks based navigation, we have previously discussed in the introductory chapters that the birds can use visual information within two different navigational strategies: the "*site specific compass orientation*" strategy (Wallraff, 1974; Holland, 2003), according to which the birds are able to recall the familiar site features in association with specific compass direction; or/and the "*piloting*" strategy (Holland, 2003), whereby the spatial relationships between the familiar landmarks are memorized by the birds to constitute a topographical representation of the familiar area.

By manipulating the bird's internal clock during clock-shift experiments, it is possible to investigate which of the two strategies the subject preferentially adopts, by observing the deviation in the birds' orientation: this deflection induced by the phase-shift manipulation is a consequence of an error in the estimation of time, which produces an error in the estimation of the sun azimuth. A preferential use of the piloting strategy will produce a marked reduction in the extent of the deflection in orientation, while a use of a site specific compass orientation strategy will be highlighted by a deviation comparable to that expected.

Several authors have investigated which factors might affect the size of deflection in orientation following clock shift. However the discrepant results in terms of the extent of the deviation in the initial orientation of the birds (Gagliardo et al., 2004), has not yet allowed to make any assumption "a priori" about the mechanism preferentially used by the birds. (Foà and Albonetti, 1980; Wiltschko and Wiltschko, 2001; Gagliardo et al., 2005b). Among the factors affecting the initial orientation of phase-shifted pigeons, it has been reported that the familiarity with the release site area strongly reduced the size of the deviation following clock shift (Bingman and Ioalè, 1989; Papi et al., 1991; Gagliardo et al., 1999; 2002; 2005b; Bonadonna et al., 2000; Holland et al., 2000; Biro et al., 2007). Another factor affecting re-orientation in clock shifted pigeons is the contemporary use of the olfactory map. In fact it has been observed that clock-shifted anosmic pigeons released at familiar sites shown a smaller deviation than their smelling companions' (Bingman and Ioalè, 1989; Gagliardo et al., 1999). A third factor playing a role in pigeons' re-orientation after clock-shift seems to depend on intrinsic features of the release site (Bonadonna et al., 2000; Gagliardo et al., 2005b).

By using GPS tracking, we have investigated which specific release sites feature might determine a more efficient re-orientation in clock shifted pigeons. To do so we trained a group of pigeons from three familiar sites located in three different directions with respect to home, we released them after clock shift and we observed the behaviour of each pigeon at each of the three sites.

MATERIALS AND METHODS

General procedure

Thirty-two pigeons (*Columba livia*, Gmelin 1789) hatched and kept at the Arnino field station (43°39′26″N, 10°18′14″E; Pisa, Italy) were used in the experiment. The experiment took place in three years (the number of pigeons used were 7, 5, and 20 in the 2009, 2010 and 2011, respectively). All the experimental releases took place under sunny conditions, with no or light wind. The pigeons were allowed to perform spontaneous flights around the loft and were kept and manipulated according to Italian law on animal welfare. About one month before the beginning of the experiments, the birds were equipped with PVC dummies, replaced with a miniature GPS data logger before the release. The miniature GPS data loggers allow the recording of the flight path with an accuracy of about 4 m (Steiner et al., 2000; Lipp et al., 2004). The position stored every second by the GPS data logger included latitude, longitude, speed and time of recording. The tracks recorded were visualized with Google Earth (Google Inc, US).

The pigeons were subjected to seven training releases in groups from each of the three sites chosen for the experimental tests (Arnaccio, home direction and distance 271°, 13.5 km; Livorno 341°, 12 km; La Sterpaia 194°, 9.5 km), plus an additional individual release from each release site. During the last training release the homing tracks were recorded with a GPS. The training phase is necessary to allow the pigeons to become familiar with the release sites and learning the topography of the over-flown area.

After the training releases, the pigeons were subjected to a fast clock shift treatment, keeping them for 6 days in a room with a light-dark cycle 6 hrs shifted with respect to the natural one. The light-tight room was ventilated by an aspirator, and it was provided with perches, food and water. The clock-shifted pigeons were then released singly from each of the three release sites, with at least 10 min between releases (to avoid pairs during the journey). For the whole period of the test releases, the birds were kept in clock-shift condition: the birds once homed were caught and put in the clock-shift room.

Quantitative analyses and statistical procedures

For the statistical analysis we have considered the tracks recorded during the eighth training release and the tracks recorded during the test release after clock-shift. We considered in this study only the birds for which the tracks from each of the three release sites were suitable for the analysis, by excluding the tracks of birds which joined during the homing flight and incomplete tracks.

For all the tracks we have considered in the analysis the position of the pigeons every 500 m at increasing distance from the release site.

With the assistance of a database available online (Astronomical Application Department of the U.S. naval Observatory), we downloaded the sun azimuth for each point of the track recorded at the clock-shifted release and we calculated the sun azimuth at the subjective time. On the basis of the sun azimuth at the time of release of each single bird, we calculated the expected deviation (i.e. the difference between the real and the subjective sun azimuth). Then we analysed separately the deviation from the home direction and that from the eighth training release track, both expressed as percentage of the expected deviation. Then, we attributed to each pigeon a score equal to the distance from the release site at which the bird turned out to be re-oriented, by assuming as re-orientation criterion a deviation lower than 25% of the expected. The differences among the reorientation distance displayed by the birds at the three release sites were compared with the Repeated Measure Analysis of Variance on Ranks (RM ANOVA on Ranks).

For each track we calculated the distance between the eighth training track and the track of the test release every 500 meters from the release site. The distance values were compared with the Two way repeated measures ANOVA.

We have considered the individual tendency of each pigeon to adopt one particular strategy on the basis of the individual score which is the sum of the distances at which re-orientation occurred at the three release sites, taking as reference the eighth training track. We considered that pigeons were applying a piloting strategy if the sum of scores was lower than 12. When the total score ranged between 12 and 24, we considered a plastic behaviour, while when the total score was greater than 24 it has been assumed that the birds are following a site specific compass orientation strategy.

RESULTS

Two out of seven, two out of five and fourteen out of twenty pigeons' tracks recorded in the 2009, 2010 and 2011, respectively, were used for the analysis. Some of the GPS data loggers (n=4) did not record or produce incomplete tracks and were therefore excluded from the analysis. Seven pigeons were excluded from the analysis because joined during the homing flight and three pigeons were lost.

Assuming as re-orientations' criterion a deviation lower than 25%, we have attributed to each pigeons a score equal to the distance from the release site at which the re-orientation occurred, as shown in the Table 1.

Considering the deviation from the home direction (expressed in percentage with respect to the expected), the comparison between the reorientation score revealed that the birds re-oriented at different distances at the three release sites (RM ANOVA on Ranks, $P \leq 0.001$). The distance at which the re-orientation occurred was significantly greater at the site from East (Arnaccio) with respect to the other two sites (Turkey test $P < 0.05$, for both comparisons). If we consider the deviation from the eighth training release tracks, the results of the re-orientation distances' analysis were the same of what has just been described for the deviation from the home directions (RM ANOVA on Ranks, $P \leq 0.001$; Turkey test $P < 0.05$, for both comparisons).

.A

Subject	Release sites		
	Arnaccio	Livorno	La Sterpaia
# 067	10	10	10
# 949	10	2	2
# 136	7	1	3
# 588	10	6	7
# 899	10	7	4
# 108	2	6	2
# 140	7	5	5
# 181	10	6	1
# 548	7	1	2
# 812	10	10	10
# 953	10	6	1
# 955	10	10	10
# 766	10	4	10
# 992	10	6	10
# c813	10	1	5
# cNCN	10	5	5
# cV	10	4	3
# c723	10	10	2

.B

Subject	Release sites		
	Arnaccio	Livorno	La Sterpaia
# 067	9	10	10
# 949	10	2	4
# 136	1	1	1
# 588	10	7	3
# 899	10	7	1
# 108	10	6	6
# 140	7	5	1
# 181	1	6	1
# 548	1	1	2
# 812	10	10	10
# 953	10	6	1
# 955	10	10	10
# 766	10	5	10
# 992	10	6	10
# c813	10	3	4
# cNCN	10	1	2
# cV	10	3	2
# c723	10	10	2

Table 1. Individual score. Distance at which the birds re-oriented at the three different release sites. A. Deviation from the home direction. B. Deviation from the eighth training release Subject: individual pigeon code number is reported; Release sites, located at East, South and North, respectively, with respect to the home loft.

Individual behaviour

From the analysis of the scores at individual level (taking as reference the eighth training track) (Fig. 1) emerged that for five pigeons the sum of the scores was greater than 24 and therefore we can assume that these pigeons consistently displayed a tendency to rely on a site specific compass strategy. For three pigeons the sum of the score was smaller than 12, we can therefore assume that they preferentially relied on a piloting strategy. All the other birds (n=10, sum score ranging from 12-24) displayed a more plastic behaviour as their changed orientation strategy depending on the release site (see Fig. 2 for examples).

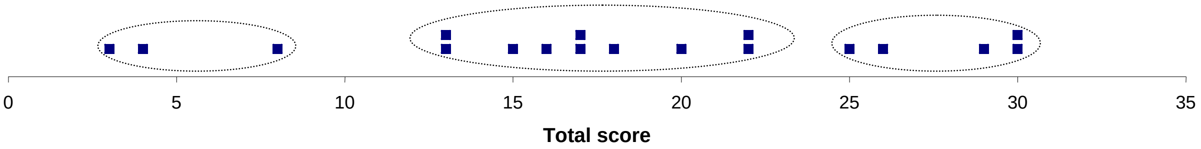

Fig. 1. Individuals total score in the analysis of the deviations with respect to the eighth training release. Explanation as in Fig. 1.

Distances between the eighth training track and the track of the test release.

The analysis about the distances between the eighth training tracks and the tracks after the clock-shift treatment at increasing distances from the release sites (every 500m, Fig. 3), revealed that there is a statistically significant difference between the three release site (Two way repeated measures ANOVA, P<0.05). In particular, the distances between the pigeons' tracks were significantly greater at Arnaccio with respect to the other two sites (Turkey test P < 0.05, for both comparisons).

The graphical representation of the mean distances between the pre and post clock-shift tracks, for the three different release sites, highlight three different trends. The birds released from Livorno have shown the tendency to follow the route followed during the last training flight after four-five kilometres from the release sites, while the birds released from La Sterpaia recapitulated their last training track soon after release. A different behaviour emerged at Arnaccio. When released at this site the birds soon diverged from the tracks recorded during the eighth training release, reorienting only further than 9 km from the release site.

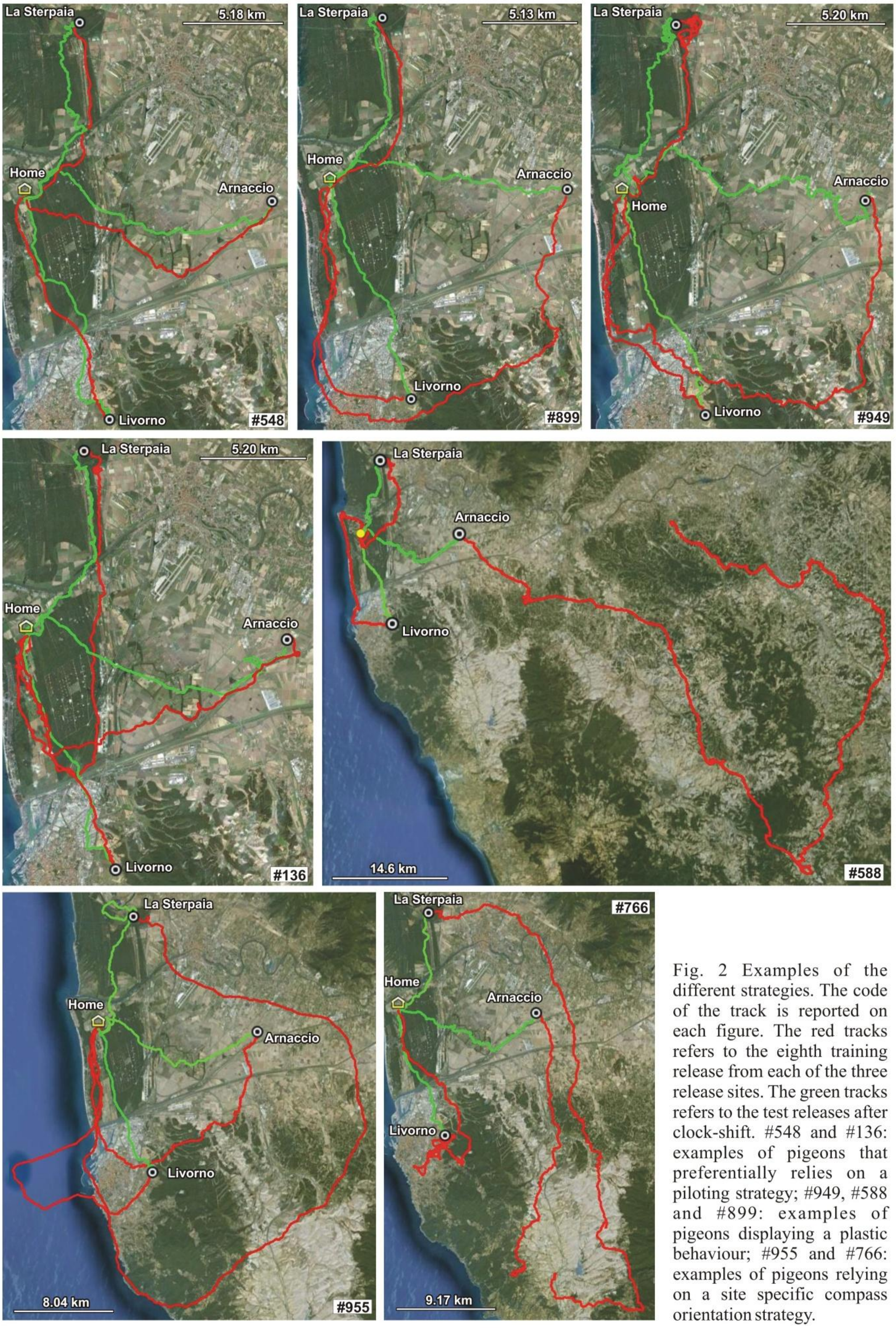

Fig. 2 Examples of the different strategies. The code of the track is reported on each figure. The red tracks refers to the eighth training release from each of the three release sites. The green tracks refers to the test releases after clock-shift. #548 and #136: examples of pigeons that preferentially relies on a piloting strategy; #949, #588 and #899: examples of pigeons displaying a plastic behaviour; #955 and #766: examples of pigeons relying on a site specific compass orientation strategy.

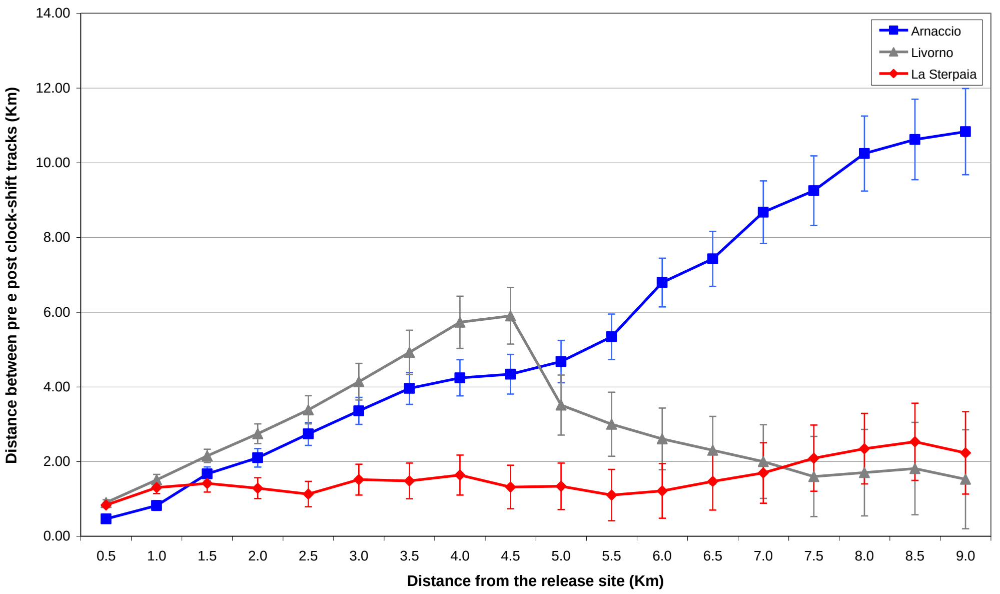

Fig. 3. Distances between the eighth training release tracks and the tracks of the test release at different distances for the three release sites (Two way RM ANOVA $p<0.05$). Standard error is represented.

Through the use of Google Earth (Google Inc, US), it is possible to visualize the single tracks of the pigeons and to observe the topography or the area at which the re-orientation occurred (Fig. 4). When released from Livorno the birds re-oriented when arriving near the sea (4-5 km distance from the release site). In fact, 8 out of 18 pigeons oriented westwards towards the coast soon after release and then following the coast line towards North, headed home. Six pigeons recapitulated their training track soon after release, while the other four pigeons (#067, #812, #955, #c723) displayed a tortuous flight path with an orientation highly variable, especially in the first part of the flight. For example the pigeons #067 and #c723 were first oriented, with a very tortuous flight path, towards South following the coastline up to 10 km from the release site. Then, heading in the opposite direction and always following the coastline, returned to home. A very curious route is that followed by the bird #812, which headed home soon after release. However, once at about 1.5 km from Arnino, instead of reaching the loft, flew towards La Sterpaia and Arnaccio before homing back. The bird #955 has flown for 6 Km over the sea to reach a rock-cliff at the Meloria Shoals and then deflected back towards the coast orienting therefore towards home (Fig.4).

At La Sterpaia, the analysis of the distances between the tracks before and after clock-shift, revealed that thirteen birds tended to re-orient toward home soon after the release, retracing the same route followed during the eighth training flight. These birds seem to be affected in their flight path by the presence of a paved road that runs along a wooded area and by the presence of agricultural areas. In this case a strong chromatic contrast appears to lead the pigeons back home. Differently from these birds, five pigeons showed a tendency to deviate toward east of about 90 degrees (with respect to the home direction), before heading back home with a circular detour.

When released from Arnaccio, only 1 out of 18 pigeon (#548) effectively retraced the last training route. The other birds progressively drifted away from the route followed during the eighth training release.

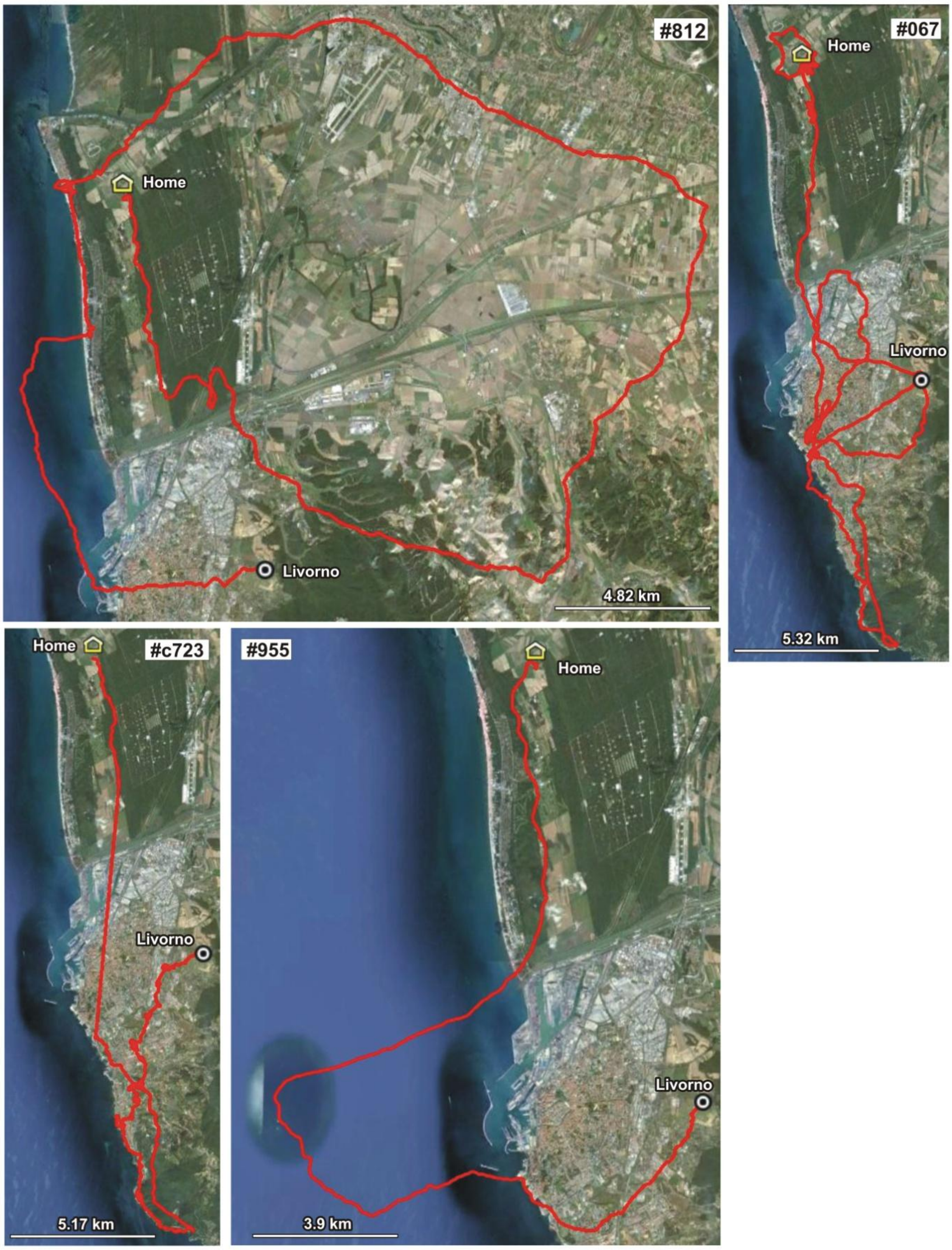

Fig. 4. Pigeon’s tracks relative to the test release from Livorno. The code of the track is reported on each figure.

DISCUSSION

This work aimed at investigating which factors might facilitate familiar landmarks-based re-orientation in homing pigeons after clock-shift. The analysis of the deviation from both the home direction and the eighth training release, revealed that the birds re-oriented at different distances from the release point depending on which site they had been released. These results are consistent with previous findings, which reported that the extent of the deviation at familiar location is site-dependent (Gagliardo et al., 2005a). In particular, the distance at which the re-orientation occurred was significantly greater at Arnaccio in comparison with the two other familiar release sites. Therefore from Arnaccio the birds seemed to have more difficulties in using a landmarks-based re-orientation strategy, and relied preferentially on a site specific compass strategy.

The analysis of the total scores at individual level highlighted a strong individual variability. All the pigeons are able to recognise the release site, but not everyone is able to learn and use the spatial relationships between landmarks to orient toward home. Three birds seemed to be able to re-orient from all the three release sites soon after release, and therefore showed to be able to use the spatial relationships among landmarks, learned during their previous homing training flights. Five birds showed poor landmark-based re-orientation abilities and showed a stereotyped behaviour relying on a site-specific compass orientation strategy. All the other pigeons exhibited a plastic behaviour, as they changed their orientation strategy depending on the release site.

In our experiment Arnaccio turned out to be the place where the use of a piloting strategy seemed to be less probable. We can explain this variability with the fact that these pigeons might be unable to re-orient on the basis of the topographical features leading the birds home where this task is more difficult. Looking at the topographic features of the three release sites, an important difference is the distance from the sea. In fact, both Livorno and La Sterpaia are quite near the sea (4.2 km and 4.5 km respectively), while at Arnaccio there is not a close view of the sea (the distance is about 14 km). The birds released from Livorno, which according to the phase shift treatment were expected to orient westward, re-oriented exactly when arriving in correspondence at the coast. In this case the coastline seemed to represent a sort of topographical barrier informing the birds about their erroneous direction. Interestingly, these results are the same obtained during an experiment conducted by training intact and HF-lesioned pigeons subjected to a phase-shift treatment (Gagliardo et al., 2009). During this experiment, the phase-shifted control birds released from Livorno recognised the sea as a salient landscape boundary: the majority of the control birds that reached the coast subsequently re-oriented northwards towards home. Therefore, the flight path of the pigeons seemed to be influenced by landscape features such as variations in the distribution of colours associated with landscape boundaries. On the contrary the HF-lesioned pigeons were diminished in their capacity to use such landscape features for navigation, in fact spent a considerable amount of time flying over the sea. These birds behaved as the birds which in our experiment do not seem able to rely on topographical features to re-orient after clock-shift, but displayed an individual and consistent preference for a site-specific compass strategy.

Interestingly, at La Sterpaia the birds displayed the best landmark-based re-orientation performances, although the expected direction after clock-shift was East, that is in the opposite direction with respect to the sea. This means that at La Sterpaia, a site where the coastline does not represent a topographical barrier because the clock-shifted birds do not tend to fly towards it, there are some features that favour an immediate re-orientation and a loyalty in retracing the route followed during the eighth training release. By looking at the tracks followed by the birds we can advance two hypotheses to explain this behaviour. The birds' flight paths at this site might be influenced by the presence of square-shaped dark green wooded areas interspersed with cultivated yellow fields. This hypothesis is supported by the tendency of some pigeons to fly along the boundaries between the two visually distinctive kinds of patches. The second hypothesis is that that the pigeons are influenced by the close view of the sea that becomes visible for a bird flying at an altitude of about 10 m.

It is worth noting that at Livorno the sea is as close as at La Sterpaia, but the behaviour of the pigeons is not identical. From the diagram (Fig.3) reporting the median deviation at the three release site, a tendency of the birds to rely on a site specific compass orientation for the first 4-5 km from the release point at Livorno, while at La Sterpaia the birds re-oriented soon after release. A possible explanation for this different behaviour might be due to the brain hemisphere engaged in processing spatially relevant visual features during the training flight. When released from La Sterpaia, the birds oriented southward during their homing flight and therefore the sea was seen with the right eye. As the fibres of the avian optic nerve cross over completely, the visual input through the right eye is mainly processed by the left brain hemisphere (Güntürkün, 1997). Therefore, it might be possible that the critical visual cues (such as the sea) might contribute more efficiently in re-orientation from a certain release site, when learned and processed by the left hemisphere. Previous studied have shown the critical involvement of the hippocampal formation in landmarks based orientation after clock-shift

(Gagliardo et al., 1999; 1996). However homing experiments performed on pigeons subjected to unilateral lesions of the hippocampal formation did not highlight any functional asymmetry in relation to the spatial use of topographical features (Gagliardo et al., 2002), as unilateral lesions turned out to impair the use of a pilotage strategy. Nevertheless, electrophysiological studies performed in freely moving pigeons in mazes have discovered peculiar proprieties of some neurons of the left hippocampus. In fact, while in both sides of the hippocampal formations there are cells sensitive to relevant and specific locations in the maze (the so called “location cells”), neurons that increase their firing activity when the bird is moving between two relevant locations inside the maze have been found only in the left hippocampus (the so called “path cells”). It has been proposed that the path cells may be more sensitive to the spatial relationship of the local visual cues with the overall spatial properties of a test environment.

As regarding the poorer ability of the pigeons to re-orient at Arnaccio, it might be due to the absence of large chromatic contrasts, as this site is a largely cultivated area, lacking of wooded patches. However, it is interestingly to note that during the training flights the pigeons seemed to be affected in their flight path by the presence of small linear landmarks as Arnaccio River, roads and short corridors delimited by aligned trees (see the birds #588 in Fig. 3). Anyway, these linear features did not seem to be sufficient to determine re-orientation in clock-shifted pigeons. In fact, all the pigeons except one, when released at Arnaccio, progressively drifted away from the route followed during the eighth training release. However, it should be noted that in previous studies it has been shown that pigeons tend to follow linear landmarks even when flying over non familiar areas. Therefore this tendency does not necessarily imply a use of linear landmarks in an allocentric spatial frame.

By concluding, these results seem to support the hypothesis that the sea might represents an important and critical topographical feature, probably due to its strong chromatic component, enhancing the ability of the birds to re-orient after a phase shift treatment. However, further and ad-hoc analysis are still required in order to better understand the role of familiar landmarks in re-orientation.

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3. CONCLUSIONS

The novel approach of this research project consisted in the investigation of old open questions by means of the use of the satellite technology. While the classical methods of investigation consisted in recording the initial orientation of birds at the release site and their homing time and success, the use of GPS loggers enabled the collection of detailed information on the behaviour of the pigeon during the entire homing journey. The analysis of the whole homing flight of birds subjected to experimental manipulations has highlighted behaviours and phenomena otherwise undetectable with traditional experiments.

As regarding the analysis of the homing flight path of experienced pigeons receiving a unilateral olfactory input, the use of GPS-technique has allowed to highlight some of the effects of the right nostril occlusion, which remained undetected when observing only the initial orientation of the birds at vanishing. In fact, while no functional asymmetry emerged in the analysis of the virtual vanishing bearings, the analysis of the whole tracks revealed that the occlusion of the right nostril affected the behaviour of the birds “en route”. Therefore a functional asymmetry in favour of the right nostril emerged in birds performing an olfactory based navigational task.

By analyzing the influence of the geomagnetic field on pigeons’ orientation, the use of GPS-loggers has allowed the comparisons of flight paths of intact controls and trigeminal sectioned pigeons studied in relation to both the geomagnetic isolines pattern and the topography of the overflown areas. The analysis of the tracks highlighted that topographical features seem to primarily affect the birds ‘routes, rather than the geomagnetic pattern, as also birds deprived of trigeminally mediated geomagnetic information, turned out to display an orientation similar to that of controls over the same terrain. In fact, the analysis revealed that the flight paths of these birds were actually shaped by the local topography in many track sections in which their orientation was also parallel or perpendicular to the magnetic slope. Therefore, it is actually more likely that the direction of the homing tracks is shaped by local topographic features, with occasional alignment of the tracks parallel or perpendicular to the magnetic slope being produced by chance.

A further progress in the analysis of the birds navigational capabilities from unfamiliar places has been possible thanks to a newly developed GPS data loggers, that allows for a remote readout of the stored data, enabling therefore the acquisition of data of birds that do not home. With this specific technology we achieved a major advance in the understanding of the role of olfactory stimuli in pigeon navigation as we could test the performances of birds made anosmic by nasal anaesthesia. By using this kind of GPS we could test the olfactory activation hypothesis that predicts that olfactory stimuli prime the navigational capabilities of birds, and that the environmental odours are solely needed to activate a navigational system that, in turn, is based on non-olfactory cues. This hypothesis challenges the olfactory navigation hypothesis, which predicts that environmental odours constitute a specific component of the navigational map in homing pigeons. Therefore, we tested experimentally whether the perception of non-sense artificial odours is sufficient to allow unimpaired navigational performances in pigeons. The analysis of the GPS tracks showed that only the birds exposed to environmental odour information displayed unimpaired navigational abilities, therefore rejecting the olfactory activation hypothesis and providing a further support to the olfactory navigation hypothesis. The use of GPS technology represented therefore a major advance in studying the navigational abilities of birds made anosmic by nasal anaesthesia, as we could observe their behaviour well beyond the vanishing bearings. In fact the observation of this parameter alone would not have highlighted the actual impairment of the birds prevented from smelling environmental odours.

As regards the investigation on the role of landmarks described in the chapter 2.4, the registration of the routes followed by the pigeons has made it possible to further investigate some aspect of pigeon’s orientation from familiar location, already described with the vanishing bearing distribution’s analysis. The analysis of the trend of the GPS tracks of each single pigeons in relationship to the characteristic features of the overflown areas, allow us to investigate which factors might facilitate familiar landmarks-based re-orientation in homing pigeons after clock-shift treatment. In fact the analysis of the tracks suggested that the sea might represent an important topographical feature, probably due to its strong chromatic component, that facilitates the ability of the birds to re-orient after a phase shift treatment. Moreover, the analysis revealed that the birds released from North, orients towards home also if the coastline does not represent a topographical barrier. In this case, other familiar release site features, as variations in the distribution of colours associated with landscape boundaries, facilitate an immediate re-orientation and a bird’s loyalty to retrace the route followed during the training release.

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