

EEG Responses to Visual Landmarks in Flying Pigeons

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Summary

Background: GPS analysis of flight trajectories of pigeons can reveal that topographic features influence their flight paths. Recording electrical brain activity that reflects attentional processing could indicate objects of interest that do not cause changes in the flight path. Therefore, we investigated whether crossing particular visual landmarks when homing from a familiar release site is associated with changes in EEG.

Results: Birds carried both data-loggers for recording GPS position and EEG during flight. First, we classified characteristic EEG frequencies of caged birds and found five main bands: A: 0–3, B: 3–12, C: 12–60, D: 60–130, and E: 130–200 Hz. We analyzed changes in these activity bands when pigeons were released over sea (a featureless environment) and over land. Passing over the coastline and other prominent landmarks produced a pattern of EEG alterations consisting of two phases: activation of EEG in the high-frequency bands (D and/or E), followed by activation of C. Overlaying the EEG activity with GPS tracks allowed us to identify topographical features of interest for the pigeons that were not recognizable by distinct changes of their flight path.

Conclusions: We provide evidence that EEG analysis can identify landmarks and objects of interest during homing. Middle-frequency activity (C) reflects visual perception of prominent landmarks, whereas activation of higher frequencies (D and E) is linked with information processing at a higher level. Activation of E bands is likely to reflect an initial process of orientation and is not necessarily linked with processing of visual information.

Introduction

The ability of homing pigeons to return from unfamiliar locations is a phenomenon for which underlying mechanisms are only partially known and still much debated. Pigeons have been shown to use the position of the sun [1–3], the Earth’s magnetic field [4, 5], and olfaction [6, 7] to home from unfamiliar places (for review, see [8]). Over familiar landscapes, they rely also on visual cues [7, 9, 10], such as highways and crossroads [11, 12]. Our previous study has shown that the ability to use such visual cues is a learned strategy [11].

The obvious flexibility of the pigeons in using different cues for successful homing poses a problem for interpreting homing data, even when data are recorded with GPS tracking. Although this technique permits a very detailed reconstruction of the bird’s flight and has allowed detection of patterns in routes such as alignment to topographical features [9, 11], the approach is limited in terms of explaining the cognitive processes behind such patterns. For example, when a pigeon does not show a change in flight path while crossing potentially interesting cues, it is impossible to recognize whether it does not perceive them or whether it chooses to ignore them by relying on other navigational cues.

A possible approach to evaluate the impact of navigational relevant cues is to analyze brain activity during flight and merge it with the GPS tracks. The goal of the present study was to provide evidence that electrical changes in the pigeon’s brain during its homeward journey can signal the visual perception of landmarks that are relevant for navigation. We expected that EEG recording could reveal reactive changes in brain activity caused by stimulus perception and subsequent processing of the information.

Attentional processing and other key functions of the brain can be analyzed by recording changes in the EEG within different frequency bands [13, 14]. To record the electrical brain activity in freely flying pigeons, we constructed “neurologgers” [15, 16], small electronic devices that are able to store EEG and neuronal activity in pigeons flying up to 100 km, recording their flight paths concurrently by miniaturized GPS loggers with a spatial accuracy of ± 5 m [17, 18] and temporal resolution of 1 s (see [Supplemental Experimental Procedures](#) and [Figure S1](#) available online), whereas the EEG was recorded from epidural electrodes.

First, we studied and characterized the EEG of pigeons in an outdoor cage while they were observing the environment. In order to recognize the EEG signatures of visual inputs, recordings were done either with both eyes open, or one eye (left or right) occluded. Because the visual pathways of the pigeon convey information from one eye preferentially to the opposite hemisphere, this procedure also served to determine the degree of asymmetrical hemispheric processing of visual stimuli [19].

In a second step, we performed a series of releases from the sea during which the pigeons initially were forced to fly over the sea (a relatively featureless visual environment) and then fly over a landscape containing familiar and navigational relevant landmarks, as evidenced by previous GPS tracking studies [11]. This served to identify different EEG signatures of information-poor and information-rich areas and to search for EEG changes when the pigeons crossed familiar landmarks.

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In a third study, the birds were released from a short distance but forced to cross two familiar landmarks. We conducted this to verify the findings from the sea releases and to analyze the electrical changes in the left and right hemisphere while the pigeons crossed such a landmark. We also released pigeons in flocks to estimate whether the diverting attention to companions and to landmarks might affect visually dependent EEG activity.

We identified prominent frequency bands, partially differing from those observed in mammals, and we could demonstrate predictable changes in the high-frequency range during release and over relevant landmarks. This permitted identification of topographical features of interest for pigeons during their homing flight.

Results

Identification of Prominent EEG Frequency Bands and Assessment of Hemispheric Asymmetries

These studies served to determine prominent frequency bands in the EEG spectrum and identify those responding to visual stimulation. Overall, analysis of the pigeon EEG revealed the following frequency bands, labeled with Latin letters from A to E for future reference: A: 0–3, B: 3–12, C: 12–60, D: 60–130, and E: 130–200 Hz (Figure 1). We avoided the use of Greek letters as commonly used in mammals, given that the interval ranges we described do not coincide with those of mammalian studies.

To determine frequency bands sensitive to visual stimulation, we housed pigeons alone in an outdoor cage and reduced their visual input by occluding with eye cups the left, the right, or both eyes. The EEG activity was recorded from four electrodes, two placed over the left and two over the right hemisphere (Figure S2). Occlusion of the contralateral eye (opposite to the recording electrodes) caused almost the same alternation of the EEG spectrum as occlusion of both eyes: a decrease of EEG power in the frequency bands 14–70 and 120–200 Hz and an increase of EEG power at other frequencies (Figure 1A). In contrast, occlusion of the ipsilateral eye (same side as the electrodes) caused much smaller variations of the spectrum (Figure 1A). The deviations were statistically significant ($p < 0.05$) in the case of both eyes occluded in bands 3–9, 18–60, 80–120, and 140–200 Hz (Figure 1B). The magnitude of changes in the left hemisphere was slightly larger than in the right (Figure S3), indicating the well-known functional asymmetry of the avian brain [20–23]. However, the EEG asymmetry in cases of symmetrical input was much smaller than the one observed after asymmetrical visual input (Figure S3). Releases of pigeons with left or right eyes occluded confirmed that asymmetrical visual input causes asymmetrical brain activation also in flight (Figure S4). Sleep in pigeons (Figures 1C and 1D) showed that the decrease of medium-to-high frequency activity (>20 Hz) may reflect their sleep state better than the occurrence of slow waves (the main mammalian indicator of sleep).

We also studied in selected pigeons the impact of neck muscle activity on EEG. Muscle artifacts were observed chiefly in the low-frequency bands A and B but were absent or negligible in the middle- and high-frequency bands (see Supplemental Results and Figure S5).

Flight over Sea and Land

The aim of these studies is to reveal the influence of presence or absence of local ground objects on EEG of flying pigeons. All pigeons had been trained previously to return from the sea with plastic dummies or GPS loggers. Birds equipped

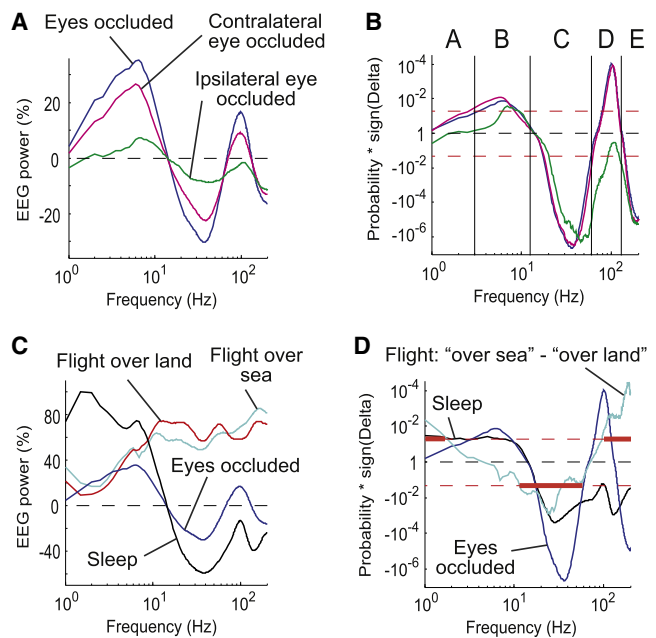


Figure 1. High-Frequency EEG Is Behavior Dependent in Pigeons

(A) Shifts in EEG power caused by eye occlusion. (B) Probability of difference multiplied by the sign of deviation. Red dotted lines mark $p = 0.05$. Letters A, B, C, D, and E denote frequency ranges 0–3, 3–12, 12–60, 60–130, 130–200 Hz, respectively. (C) Deviations in EEG power of four different states: eyes occluded, sleep, flight over land, and flight over sea. (D) Statistical significance of deviations in sleep and with both eyes occluded from the open-eyes state; significance of difference between flights over sea and over land.

with EEG and GPS dataloggers were then released over the Mediterranean Sea—providing a visually featureless environment—at a distance of 18 km from the coast, ~30 km from their loft (Figure 2). Three releases were performed in 1 week intervals. The visibility between these releases was different, ranging from poor to excellent. The average duration of such flights was 37.8 ± 11.7 min (mean \pm SD), and flight speed varied between 60 and 80 km/hr, indicating that the additional weight of the EEG datalogger carried by the pigeons was no handicap. Further details can be found in the Supplemental Results and Figures S6 and S7.

In order to visualize the dynamics of EEG changes, we color-coded GPS recorded flight segments of 1 s duration (~16–22 m depending on speed) in sequential dots showing the deviation from the average power of a given frequency band. In comparison to the resting state, the EEG power during flight (both over sea and land) increased relative to the resting state (+60%–80%) in the frequency ranges of 10–200 Hz (Figure 1C). However, over sea the power of the middle frequencies (12–60 Hz) was diminished, whereas both very low (0–2 Hz) and high (100–200 Hz) frequencies were increased (Figures 1C and 1D). These intervals approximately coincide with intervals previously labeled C, A, and E, respectively. In the frequency bands C and E, variations of power were not correlated.

Variations in EEG Power when Flying over Sea

The color-coded trajectories in Figure 2 show that the high EEG power in C and E frequencies near the release site decreased with time after release. Quantitative analysis of EEG in the

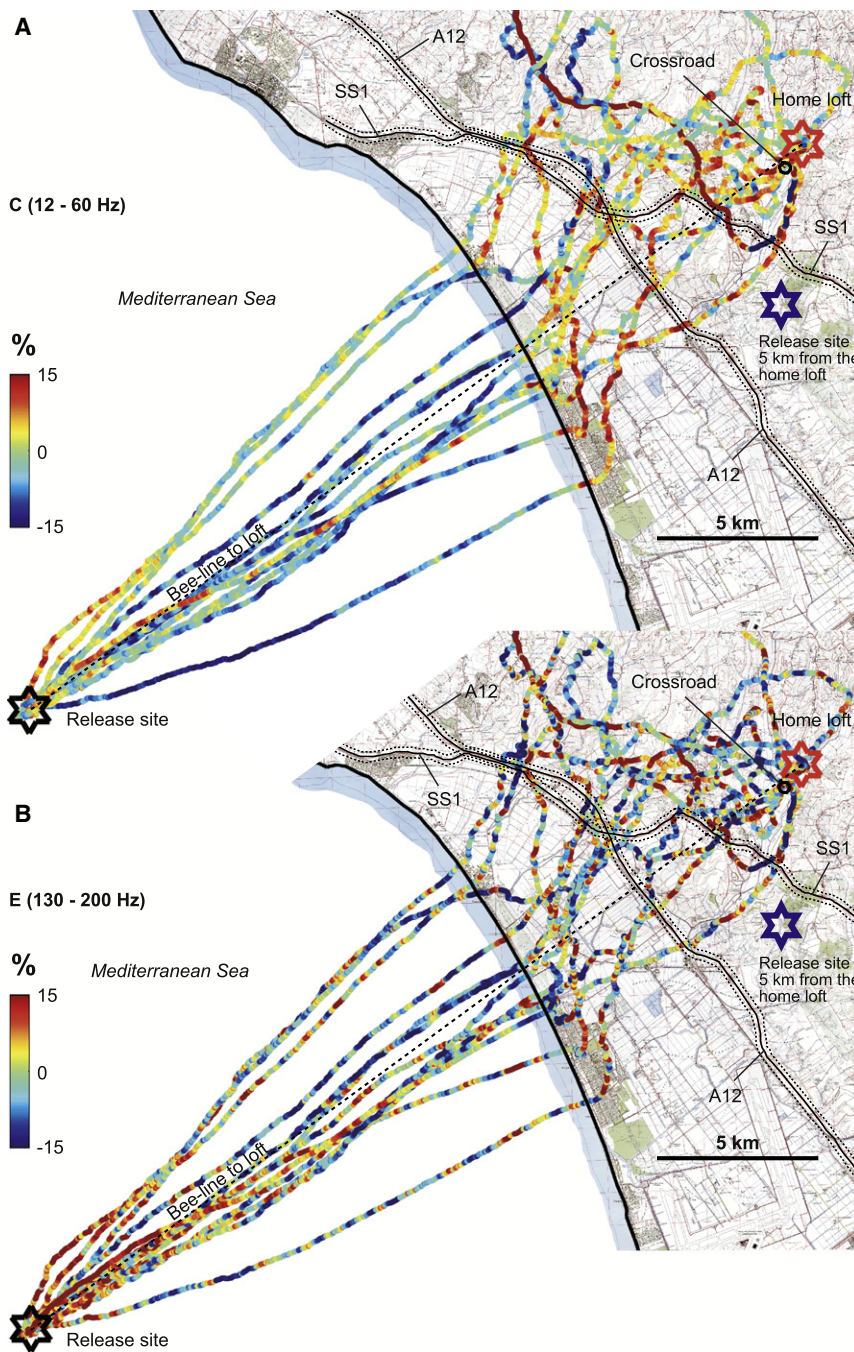


Figure 2. EEG in Pigeons Differs in Flight over Sea and over Land

(A) shows a color-coded representation of deviation of C band (12–60 Hz) EEG power from the average during the flight. (B) shows a representation similar to (A) for E band (130–200 Hz). EEG power in the 12–60 Hz frequency range was much higher over land than over sea. In the frequency range 130–200 Hz, the ratio was the opposite (EEG power over sea was higher than over land). Both differences were statistically significant, with the maximum statistical difference $p = 1.3 \times 10^{-3}$ reached at $f = 25$ Hz and $p = 3.45 \times 10^{-5}$ reached at $f = 185$ Hz for the first and the second frequency bands, respectively (Figure 1D, nonpaired two-tailed t test, $n = 13$). It should be noted that EEG power in both frequency ranges sometimes increased when animals crossed longitudinal features like the coastal line and highways A12 and SS1 along the coast. These highways have been observed to be used by pigeons as guidance from NW to the loft [11]. Dotted lines along the highways mark 150 m areas near them.

releases under different visibility showed some subtle but significant differences in the frequency ranges C and D, but none for frequency range E. The observed differences were possibly related to visibility, flight experience, and motivational status (see Supplemental Results and Figures S6–S8).

Variations in EEG Power when Flying over Land: Increase in C Frequency Range Occur over Landmarks

Figures 3A–3C show a clear peak of activity in the C band when pigeons crossed the coastline. To check whether such activation occurred also over other main landmarks, we analyzed the changes in the C band while the pigeons were crossing other familiar landmarks known to be followed by pigeons from earlier studies: i.e., the highways A12 and SS1 (“Aurelia”) and a point passed by many pigeons just before arriving at the loft (“crossroad”). These locations are marked in Figure 2. We calculated the EEG power in an area of ± 150 m from the landmark

frequency bands C, D, and E revealed a linear decrease with distance from the release site (up to 1 km before the coast) as evident in power density (Figures 3A and 3B) and power (Figure 3C). Afterward, values rose again. The decline in EEG power was $0.23\%/km \pm 0.51\%/km$, $0.51\%/km \pm 0.48\%/km$, and $0.67\%/km \pm 0.91\%/km$ (mean \pm SD) for the C, D, and E frequency ranges, respectively (Figure 3D). The decline in all three ranges was statistically significant ($p = 0.0109$, $p < 0.0001$, and $p < 0.001$, $n = 33$, two-tailed t test). However, these values were not decreasing with a similar rate. This implies again that oscillations at these frequencies reflected different brain processes that were not necessarily linked with one another. Although Figure 2 shows the results from a release with excellent visibility only, a detailed comparison of the three

that approximately coincides with the width of the peak of EEG activation over the coastline in Figure 3C. The borders of these areas are labeled by dotted lines along highways and by a circle ($d = 300$ m) over the crossroad in Figure 2. Seventeen tracks (out of 33) crossed the area of the circle. To check whether changes in the EEG over such locations occurred by chance, we used a bootstrap simulation method that shifted randomly all short EEG epochs of 1 s along the trajectory over land and calculated the probability that an observed local change in EEG power would coincide by chance with the selected locations. For details of the bootstrap simulation, see Supplemental Experimental Procedures.

The highway SS1 was crossed by 32 tracks, given that one pigeon stopped before and the battery of its GPS logger was

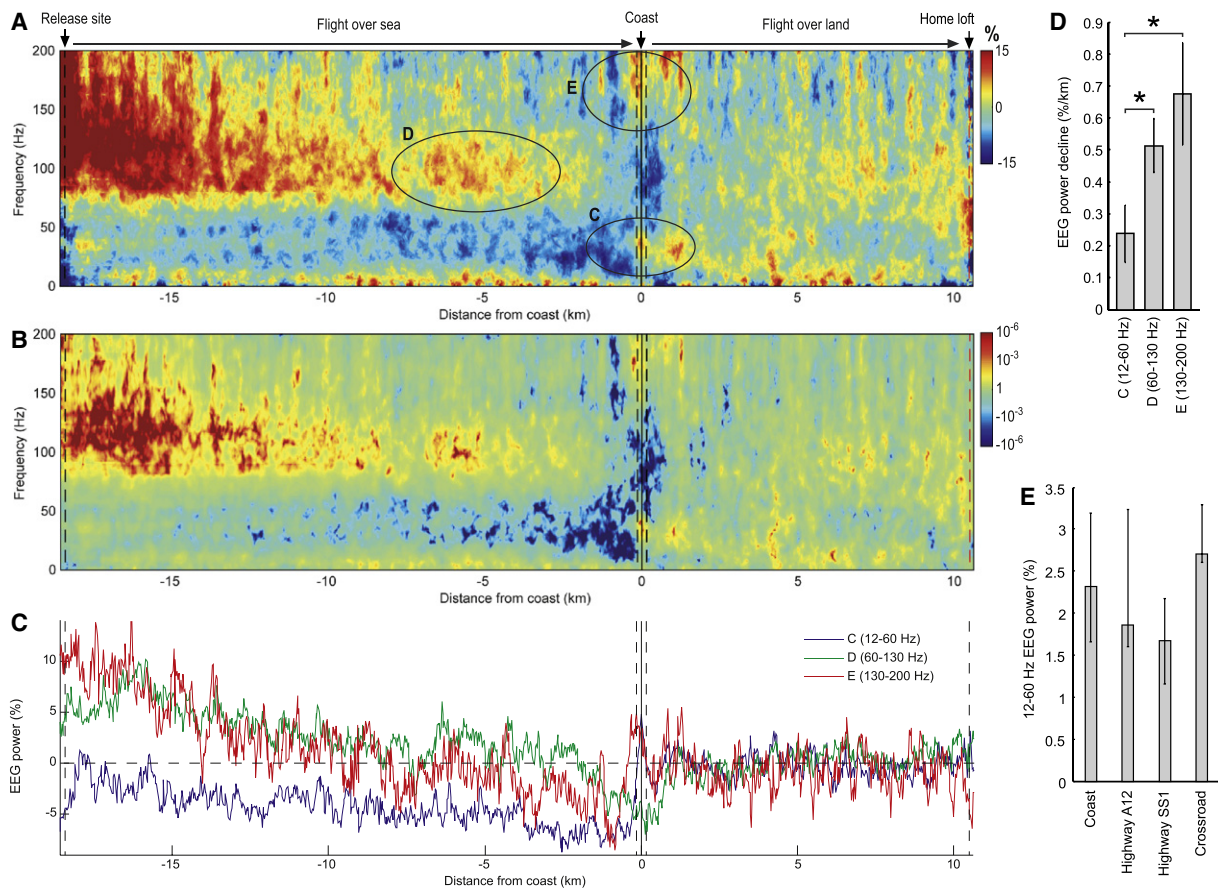


Figure 3. Dynamics of EEG Power over Sea and Coast

(A) Distance frequency (DF) representation of EEG power (in percentage from average EEG power over land). Ovals emphasize separate activations in three frequency bands C (12–60 Hz), D (60–130 Hz), and E (130–200 Hz). Dotted lines near the coast mark 150 m area near it. The horizontal scale indicates distance to the coast.
 (B) DF representation of probability (bootstrap, 33 tracks) multiplied by the sign of deviation.
 (C) Dynamics of EEG power in three frequency bands C, D, and E. Note a very sharp peak of activity in the C band just over the coastal line. Activation of the E band foreruns it.
 (D) Decline of EEG power over sea takes place in all frequency bands C, D, and E (mean \pm SEM). However, the rate of decline is different for different frequencies. * $p = 0.005$ (paired two-tailed t test, $n = 33$).
 (E) Activation of the C band takes place at all main landmarks on pigeons' way home: coastal line, highway A12, highway SS1, and crossroad just near the home loft (median). All landmarks are plotted at the map in Figure 2. Error bars show quartiles for the medians.

discharged. All 33 tracks with color-coded EEG power of the C band are plotted together in Figure S9. Clearly, in many tracks EEG activity was increased over these landmarks. A detailed quantitative analysis of changes in the C frequency band power is given in Figure S10. In brief, C band EEG activation, measured as average in a 150 m zone around the landmarks, was statistically significant when crossing any of these locations ($p < 0.01$ for the coast, $p < 0.0001$ for highway A12, $p < 0.001$ for highway SS1, and $p < 0.000001$ for the crossroad, as estimated by bootstrap simulation). These were not the only locations eliciting activation of the C band but the ones with the highest predictability.

Activation of C Frequency Range Is Lower in Flock-Flying Pigeons

To analyze location-dependent C band activation with more replicates, we released pigeons from a distance of 5 km from the home loft. We chose the release site (Figure 2) to force the pigeons to pass two landmarks close to the home loft, namely the highway SS1 and the small crossroad near the

home loft (see satellite aerial photos of the area and landmarks in Figures S11 and S12). In order to vary the degree of visual attention paid to these landmarks, we released the birds either singly (48 tracks, Figure 4A) or in flocks of six (54 tracks with good EEG, ten flock releases, Figure 4B). Because pigeons must divert their visual attention to their companions in order to stay in the flock, we expected them to pay less attention to landmarks.

Single birds flew significantly slower than flocks (8.3 ± 2.4 versus 5.6 ± 0.4 min (mean \pm SD; $p < 0.00001$, $N_1 = 48$, $N_2 = 10$, Mann-Whitney). EEG power increased ~ 150 m before the ground features of interest (highway or crossroad) and decreased 150 m after it (Figures 4C and 4D). This was true for both single- and group-released birds over the SS Aurelia (Figure 4E, for single releases $p < 0.0000001$; for flocks $p < 0.001$) and the crossroad (Figure 4E, $p < 0.0001$ and $p < 0.0001$, respectively). The lower increase of EEG power in the flock-flying pigeons was verified by bootstrap simulation ($p = 0.045$, Figure 4E), confirming our hypothesis of visual attention diverted to companions. On the other hand, the increase of

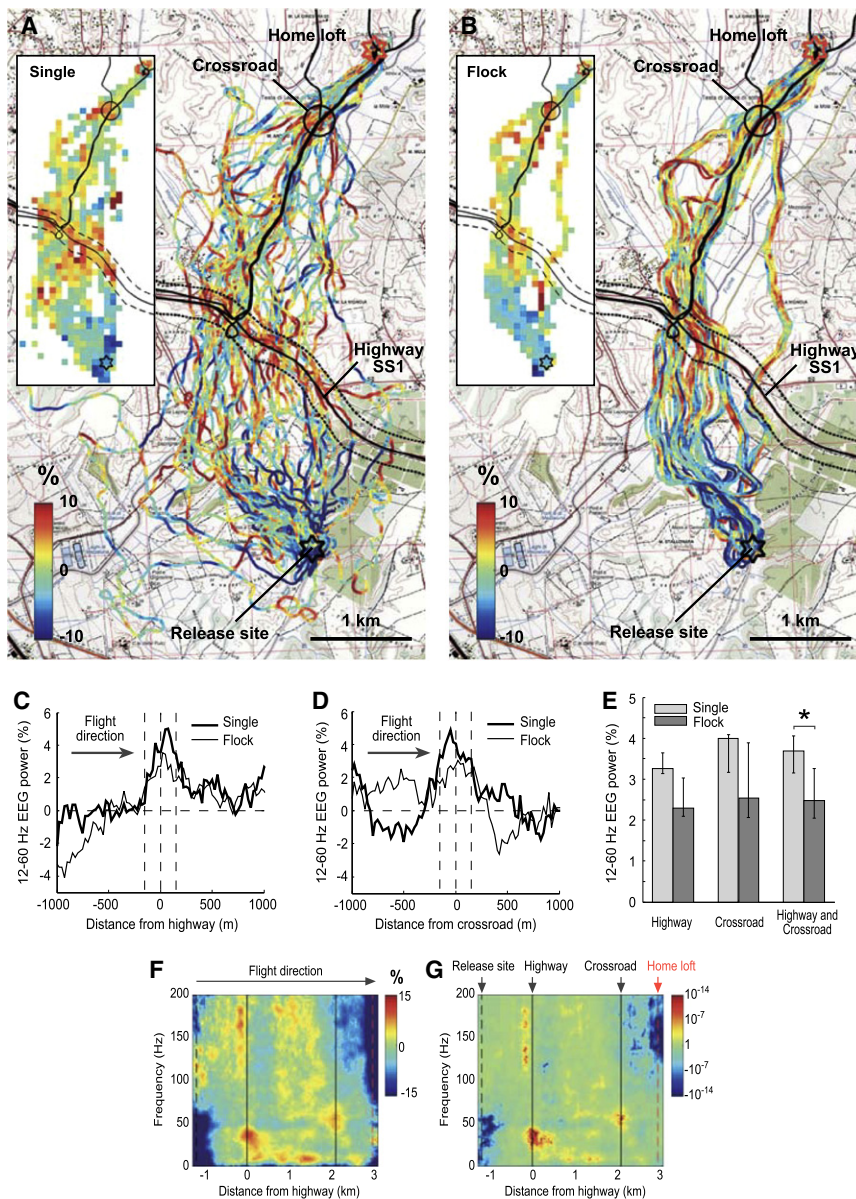


Figure 4. Twelve to Sixty Hertz EEG Power Is Increased in Pigeons Flying Singly and in Flock over Navigation-Relevant Landmarks such as Highway and Crossroad

(A and B) C range (12–60 Hz) EEG power in pigeons flying alone (A) and flying in flock (B). Trajectories of individual flights are plotted over a topographic map. Colored segments indicated deviations from the average of EEG power in C (12–60 Hz) range in percents from this average. Average values were calculated individually for each flight for removal of influence of intersubject and day-to-day variability. The circle around the crossroad and two dotted lines near the highway mark a 150 m zone near the landmarks. Insets show EEG power averaged in 100×100 m squares (deviation in percentages). Only squares containing at least three trajectories are shown. Note the increase of EEG power—marked in orange—over the landmarks, especially in pigeons flying alone.

(C and D) Dynamics of C range (12–60 Hz) EEG power near the highway and crossroad.

(E) C range EEG power in 150 m zone near the landmarks. Error bars show quartiles for the medians. * $p = 0.05$ (bootstrap, 22 birds).

(F) Distance frequency (DF) representation of EEG power (in percentages). Note maximum EEG power in the E range 150 m before the highway.

(G) DF representation of probability (bootstrap, 22 birds) multiplied by the sign of deviation.

EEG power over the highway and the crossroad had significant variance (deviation from baseline in percents of baseline EEG power—over highway: $3.0\% \pm 3.9\%$, over crossroad: $4.5\% \pm 9.7\%$, mean \pm SD) and did not occur in all pigeons, or varied with the day of release, particularly near the crossroad. Consistent with the in-cage studies with eye occlusions, landmarks lying asymmetrically to the pigeon path caused stronger activation in the contralateral (opposite to the object) hemisphere. For details, see Supplemental Results and Figures S13–S16.

Can EEG Analysis during Flight Detect Objects or Locations of Interest for Pigeons but Not for Experimenters?

Given that the EEG activation near the crossroad immediately before the loft did not make sense in terms of navigational landmarks, we conducted a detailed analysis of left- and right-hemispheric EEG responses at this place and at another location near the loft marked by a spot of EEG activity in a majority of birds passing there (see insets of Figures 4A

and 5). A high-resolution satellite picture showed that there was a small valley with a farm and a cattle paddock (Figure S17). Interestingly, the increase in C band activity appeared more pronounced in the right hemisphere of two birds passing to the right of the farm, yet more pronounced in the left hemisphere of the three birds passing the farm at left (Figure S17). This indicates separate activation of the EEG through the eye that first perceived the farm while passing. Using bootstrap simulation, we estimated post hoc the probability

to get the measured degree of activation in the area of four squares (400 m^2 , labeled “1” in Figure 5A), similarly to what we did in 150 m ranges near other landmarks. This area was crossed by six tracks. In the left hemisphere, the C band showed significant activation ($10.5\% \pm 4.6\%$, mean \pm SEM $p = 0.022$), but not in the right hemisphere ($3.7\% \pm 3.0\%$, $p = 0.22$). The average activation of both hemispheres was $7.65\% \pm 3.3\%$, $p = 0.021$.

Likewise, a comparison of the activity of left and right hemispheres in tracks near the crossroad (Figures 5A and 5B) showed that pigeons passing the crossroad at left had left-hemispheric activation and those flying just above it a right-hemispheric activation (area is labeled as “2” in the inset of Figure 5A). This implies that the real object of interest was not the crossroad itself but a nearby object, possibly a barn located 60 m to the left of the crossroad (see also Figure S12B).

The riddle of such highly specific C band activation found in places close to the loft and likely to be irrelevant for proper navigation was then elucidated by visiting these places: both

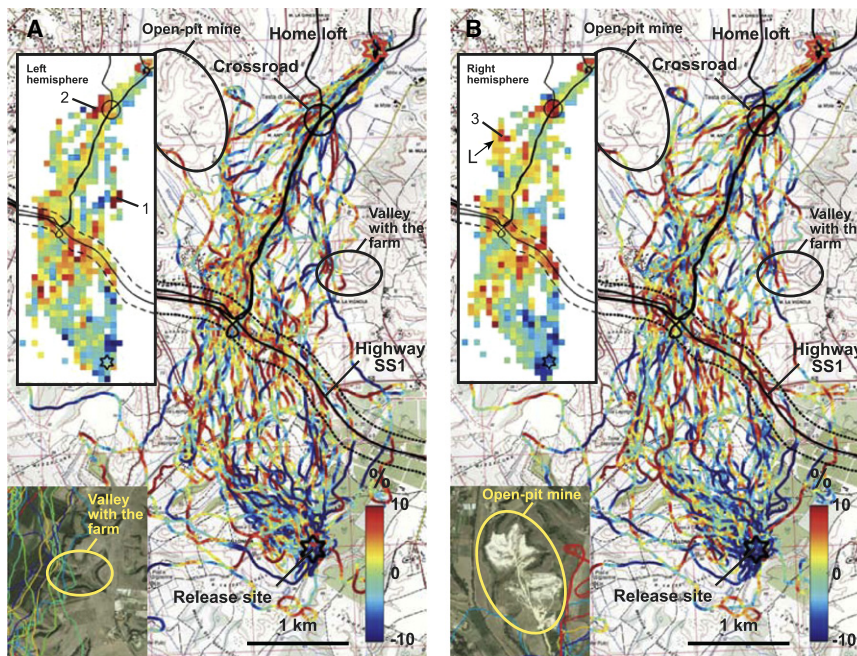
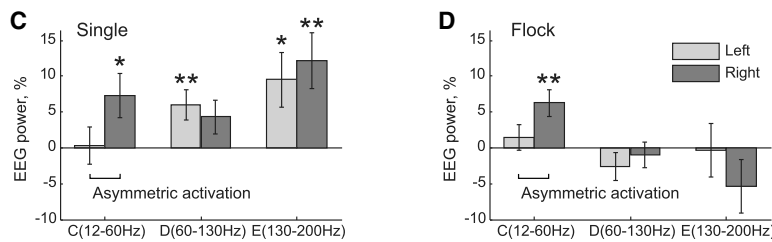


Figure 5. Changes in EEG Power in the Left and Right Hemispheres in Pigeons over Landmarks (A) Map and color-coded C range (12–60 Hz) EEG power in the left hemisphere (in percentages) in pigeons flying alone. (B) The same in the right hemisphere. For explanations, see legend Figure 4 in which similar panels are shown. Note the asymmetrical brain activation over areas labeled 1 and 2 in the inset of (A), suggesting that the place of interest was at the right side of the pigeon's track. Asymmetrical brain activation was also observed over the area labeled 3 in the inset of (B). This activation was most probably caused by well-visible open-pit mine at the left (see photo inset of B and Figure S11). Some increase of path entropy [24] in this area (Figure S13) can be explained by this landmark. (C) Changes in EEG power near the open-pit mine in pigeons flying alone. (D) The same in pigeons flying in flocks. Asterisks mark significance of deviations from zero. * $p < 0.05$, ** $p < 0.005$, bootstrap. Error bars show standard error of the mean.



This corresponds to a size of a 100×100 m square measured diagonally (141 m). Thus, the area where D and E band activation can be expected is a single-layer array of squares to be crossed by a pigeon just before the area of C band activation. In this case, it consists of four squares arranged in the form of the letter “L.” The letter is shown in the inset of Figure 5B; the arrow points at the array

harbored a colony of feral pigeons; that is, these were places of high ethological relevance for our birds.

Can EEG Changes during Flight Be Linked to Directional Changes of the Flight Path?

In order to evaluate the usefulness of EEG changes for the analysis of spatial orientation processes, we searched for changes in flight path suggestive of reorientation toward a new goal. One such site was a bright and well-visible open-pit mine labeled as “3” in the inset of Figure 5B. The mine extended into a valley leading away from the loft. The trajectories of the flight paths (see Figure S11) suggested that several pigeons were first attracted by the mine but reoriented subsequently toward the loft. We first analyzed possible changes in the C band to estimate the degree of visual attention paid to this landmark and, afterward, changes in the D and E bands suspected to be indicators of course setting during the sea releases.

Six tracks passed through this area, all to the left of the open-pit mine. Remarkably, the activation of the C band in the area of three squares (300 m^2 , label “3”) was absent in the left hemisphere ($p = 0.91$), but present in the right ($p = 0.020$; Figure 5C). The averaged activation of both hemispheres was not significant ($4.0\% \pm 2.46\%$, $p = 0.10$). Thus, passing this well-visible object seen by the left eye probably caused a predominant activation of the right hemisphere.

From the previous analysis, it was known that C band activation can follow activation of D and E bands after a relatively small distance of ~ 150 m (Figures 4F and 4G and Figure S14).

location. Ten tracks of individually released birds crossed this area. Indeed, both D and E bands were activated in this L-shaped area in either hemisphere (Figure 5C). The probabilities of deviations were: D band left, $p = 0.0045$; D band right, $p = 0.060$; D band total: $5.52\% \pm 1.95\%$, $p = 0.0046$. Similarly for E band: $p = 0.012$; $p = 0.0016$; total $10.57\% \pm 3.39\%$, $p = 0.0018$.

Two flocks of six pigeons also flew over the area labeled “3” and over the adjacent L-shaped area, providing 11 tracks with complete EEG recordings. Activation of the C band was similar to activity in birds flying along: the left hemisphere was not activated significantly ($p = 0.39$), and the right hemisphere was strongly activated ($p = 0.00052$), leading also to significant average overall activation $3.64\% \pm 1.49\%$, $p = 0.0145$ (Figure 5D). Notably, these estimates are not post hoc because the areas were determined from a previous independent data set. Contrary to birds flying alone, no activation of D and E bands was observed in flocks (Figure 5D): D band left: $p = 0.19$, D band right: $p = 0.58$, D band total: $-1.76\% \pm 1.48\%$, $p = 0.23$. Similarly for the E band: $p = 0.93$; $p = 0.15$; total $-2.55\% \pm 3.15\%$, $p = 0.42$. Activation of D and E bands was also not detected on tracks before the L-shaped area.

Alternations in EEG over essential landmarks were also accompanied by decrease of speed and increase of the path entropy [24], a measure of stochasticity of the trajectory (see Supplemental Results and Figure S18). However, these parameters could not indicate the location of the object of interest as clearly as it is seen from C band EEG activation (see Supplemental Results and Figure S19).

Table 1. Activation of Frequency Bands at Release Sites and Landmarks

Experiment	Release Site	Coast	Highway A12	Highway SS1	Open-Pit Mine	Crossroad
Sea releases (~30 km)	C~, D↑, E↑	C↑, D↓, E↑	C↑, D↑, E~	C↑, D↑, E~	—	C↑, D↑, E~
Land releases, in flocks (5 km)	C~, D↑, E↑	—	—	C↑, D↑, E↑	C↑, D~, E~	C↑, D↑, E~
Land releases, alone (5 km)	C~, D~, E~	—	—	C↑, D↑, E↑	C↑, D↑, E↑	C↑, D↑, E~

Discussion

This study represents the first recording and analysis of electrical brain activity temporally integrated with large-scale navigational movements in free-flying birds. The changes of activity in revealed frequency bands C (12–60 Hz), D (60–130 Hz), and E (130–200 Hz) in relation to release site and landmarks are summarized in Table 1. C band was activated always at landmarks or places of high ethological interest, but never at release sites. Its activation could be unihemispheric. The D band appeared activated when a flock was formed at sea release sites and at all landmarks except the open-pit mine in flock flyers. The E band was activated when a flock was formed at sea release sites, at the first prominent landmark on the pigeon's way after release, or when direction of the flight was changed at the open-pit mine.

Whereas the C frequency band appears to be mostly associated with the visual perception of significant landmarks (both navigational and ethological), the E frequency band seems to be activated at moments when some additional cognitive processing is taking place. Such cognitive processing might be needed for initial orientation at sea release site when no local landmarks are present, and the analysis of sensory signals is required for large-scale navigation ("sun compass," earth magnetic field, olfaction). Also, when the first significant familiar landmark appears on the pigeon's way, some adjustment of direction by this cue may be associated with E band activation. The D frequency band appears somewhat transitional between E and C and requires additional analysis (see also Supplemental Discussion for more details).

To summarize, this study has shown that the analysis of power changes in high-frequency bands of the pigeon EEG can clearly identify (previously known) visual navigational landmarks, but also ethologically relevant places. Therefore, we expect that changes in the EEG of flying pigeons will also be helpful in detecting and analyzing the navigational impact of local geophysical or airborne stimuli.

Experimental Procedures

Study Area and Facilities

Homing pigeons were kept in the facilities of the University of Zurich at Testa di Lepre, Italy, 25 km NW of Rome (12.27930° N; 41.93261° E). All birds underwent regular training in small flocks or individually from all directions within 50 km from the loft.

Subjects

Twenty-six 2- to 3-year-old pigeons of both sexes were used. All procedures were approved by the Swiss (Cantonal Veterinary Office) and Italian authorities (Ministero della Salute and Istituto Superiore di Sanità, D.L. vo 116 del 27/01/92) for animal welfare.

Surgery

Gold-covered watch screws were used for epidural EEG recording.

Data Acquisition

EEG and EMG were amplified, filtered, digitized by the Neurologger (<http://www.vyssotski.ch/neurologger2>) and stored in its memory with a rate of 400 samples per second. Starting time was recorded with the accuracy of 1 s

synchronization with the GPS data. Downloaded data from both loggers were analyzed with the MATLAB environment (MathWorks, Natick, MA, USA) with the help of custom written scripts and EEGLAB package [25].

GPS Recordings

Between releases, birds wore PVC dummies of the same weight as the GPS in order to habituate them to the load. The total load did not exceed 30 g. For experiments, the dummies were replaced with GPS-loggers (Newbehavior, Zurich, Switzerland; Technosmart, Rome, Italy) just before the release and placed again on the bird after retrieving the GPS at the loft. Pigeons were transported to the release site by car in a well-ventilated, visually shielded transport cage. Prior to release, GPS loggers and neurologgers were activated, and the birds were placed individually in a small releasing crate permitting scanning of the environment. They were not tossed but allowed to leave the crate after opening of the cover. This helped to estimate the flight motivation of the pigeon.

Supplemental Data

Supplemental Data include 20 figures, Supplemental Results, Supplemental Experimental Procedures, one table, and Supplemental Discussion and can be found with this article online at [http://www.cell.com/current-biology/supplemental/S0960-9822\(09\)01250-0](http://www.cell.com/current-biology/supplemental/S0960-9822(09)01250-0).

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