Pigeons with ablated pyriform cortex home from familiar but not from unfamiliar sites

(bird navigation/brain lesions/olfaction)

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ABSTRACT Homing behavior was tested in pigeons (*Columba livia*) after removing a portion of the ventrolateral telencephalon, which receives extensive projections from the olfactory bulb and is comparable with the mammalian pyriform cortex. Ablated pigeons show unaffected cardiac responses to odorous stimuli but altered homing behavior. After the operation, the birds were trained by repeated flock releases along with control birds from a site 40 km from the loft. After being released singly from this familiar site, the ablated birds turned out to be unaffected by the operation. In releases from two unfamiliar sites, ablated birds, unlike control birds, were not homeward oriented and were mostly lost. The ablation of the pyriform cortex has the same effect on homing behavior as olfactory deprivation. It can be concluded that the pyriform cortex plays an important role in the specific mechanisms linking olfactory inputs with the navigational response.

In recent years an increasing number of studies have led to a better understanding of the mechanisms that allow pigeons to home and of how they acquire this ability. This achievement has stimulated neurobiologists to plan and perform the first steps in the study of the neurobiological basis of homing behavior.

Pigeons are known to rely on two homing mechanisms. The first operates over familiar and unfamiliar areas and is based on a navigational map combined with a sun or magnetic compass. As shown by a number of studies, the navigational map can be acquired at the home loft by associating wind-borne odors with the direction from which they come. Once birds have a fully developed map, they can establish their position by smelling local atmospheric odors and fly in the deduced direction by means of a compass. Anosmic pigeons show a severely impaired ability to home from unfamiliar sites, in terms of initial orientation and homing success (see refs. 1–5 for reviews). Some authors, however, maintain different opinions on the homing mechanism operating over unfamiliar sites (6, 7). Alternative hypotheses deal with the possibility that the earth's magnetic field is the basis of a navigational map (8–10) or is used to establish the direction and distance of displacement during passive transport (11).

The second homing mechanism is a nonolfactory pilotage based on familiar landmarks; it enables pigeons to home from previously visited sites even when they have been made anosmic (12–14). This shows that the birds can also acquire a "topographical map" that is independent of the navigational (olfactory) map.

To investigate the nervous mechanisms underlying homing, a series of homing tests following telencephalic ablations has been undertaken. Ablation of the anterior forebrain (Wulst) does not impair homing from familiar and unfamiliar sites (15, 16), whereas ablation of the dorsomedial forebrain (hippocampus and parahippocampus region) produces a deficit in the use of a spatial memory relative to familiar landmarks. Therefore even when operated birds are released from familiar sites, they have to rely on their navigational (olfactory) map to reach the home area and, once there, appear to be unable to recognize their loft. When made anosmic, these birds show an impaired capacity for homing, even from familiar areas (for a review, see ref. 17).

No brain center has thus far been shown to be involved in the navigational mechanism that allows homing from unfamiliar sites. In the present paper, we report the results obtained by testing the homing ability from familiar and unfamiliar sites in pigeons subjected to ablation of a portion of the ventrolateral telencephalon that is recognized to be comparable with the mammalian pyriform cortex (18). We chose this area since it receives extensive bilateral projections from the olfactory bulb (19) and may therefore be involved in olfactory information processing. To complete our study, pyriform cortex-ablated pigeons were also tested for olfactory-induced visceral responses.

MATERIALS AND METHODS

Birds and Releases. The homing pigeons (*Columba livia*) used in this study were all housed in a loft at Arnino near Pisa. At about 3 months of age they were arbitrarily subdivided into intact control (C) birds and ablated experimental (E) birds. The E birds underwent ablation of the pyriform cortex (CPi in the pigeon brain atlas; ref. 20) on July 13–15, 1988. After the operations, all of the birds were released as a flock, first from two intermediate sites (16.7 and 26 km, August 12 and 16, respectively) and then from the release site destined to become familiar, which was 40.4 km NNW of the loft (five flock releases before the end of August). Five E birds of 30 and 3 C birds of 26 did not home from training flights.

In the critical releases, the birds were tossed singly from the hand, with alternation between the groups, and were followed with 10 × 50 binoculars until they disappeared from view. From the vanishing bearings, the mean vector direction and length were calculated by vector analysis (21) for each group. Bearing sets were tested for randomness by the Rayleigh test and compared by Watson's U^2 test; bearing clustering around the home direction was subjected to the V test (21). Comparison between vanishing intervals and between homing performances in the single experiments was carried out using the Mann–Whitney U test (22).

Arrivals at the loft were recorded continuously during release day.

The critical releases took place as follows:

(*i*) Aug. 30. From the familiar site (Massa), home distance 40.4 km, home direction 161°. Twenty-two C and 22 E birds were released.

Abbreviations: CPi, pyriform cortex; C, control; E, experimental.
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(ii) Sept. 2 and 6. From a first unfamiliar site (Villamagna), home distance 44.5 km, home direction 298°. The release, begun on Sept. 2, was interrupted because of the changing weather and was completed 4 days later. Ten C and 12 E birds were released. Except 1 E bird, they had all taken part in the Aug. 30 release from the familiar site.

(iii) Sept. 6. From a second unfamiliar site (Bolgheri), home distance 54.8 km, home direction 336°. Twelve C and 10 E birds were released. Their only previous test release had been that of Aug. 30, except that 1 C and 1 E bird had also been released on Sept. 2, and 1 C and 1 E bird had only been released for training.

All of the releases were performed with the sun disc visible and with no or light wind.

Surgical Procedures. For ablations, pigeons were anesthetized with chloral hydrate (20%) in Ringer's solution for birds (0.25 ml/100 g of body weight) and placed in a stereotaxic apparatus. Lesions were made bilaterally by aspiration through a pipette and under microscopic viewing. Target coordinates were obtained from the stereotaxic atlas of the pigeon brain (20) and were restricted to the CPi region. They extended from anterior 7.50 to anterior 5.00 at a depth of 0.5–1 mm from the pial surface.

Almost all of the birds recovered well from surgery. We could discern no qualitative differences in the general behavior of E birds compared with that of the intact C birds.

Histology. An anatomical check on the lesions was carried out on six pigeons who homed after the critical releases and on six pigeons not used in the homing experiments. Pigeons were deeply anesthetized with cloral hydrate and perfused via the left ventricle with 10% formalin. Once removed, the

brains were cut on a coronal plane into 30-μm sections, and the sections were then stained with cresyl violet. With the aid of a microscope microprojector, the lesions were plotted on coronal standard sections of the pigeon brain derived from the pigeon brain atlas (20). Figs. 1 and 2 present lesion plots for four pigeons. The sample consists of two birds not used in the homing experiments (Fig. 1) and two birds that returned to their lofts after the experimental releases (Fig. 2). As can be seen, lesions consistently involved the CPi on both sides of the brain and were associated with small and varying amounts of damage to adjacent structures, including area temporo-parieto-occipitalis, area corticoidea dorsolateralis, neostriatum, archistriatum, archistriatum pars dorsalis, and archistriatum pars ventralis. Comparisons show only minor differences between birds not used in the experiments and birds that had homed from the test releases.

Visceral Responses. Visceral responses were evaluated by measuring the changes in heart rate that occur upon the presentation of odorous stimuli. Four CPi-ablated pigeons (not used in the homing experiments) and six controls were used. Olfactory stimulation and electrocardiogram recording were performed according a previous paper (23). The animals were tested 1 day before and 6 days after the E birds' CPi ablation. Cardiac responses to the stimulus were evaluated by comparing the heart rate in the 30 sec preceding the stimulation and the 30 sec following it. Levels of significance were assessed with the Student's t test.

RESULTS

Homing Behavior. After being tossed at the familiar site, many birds landed or perched in sight of the observers, so that
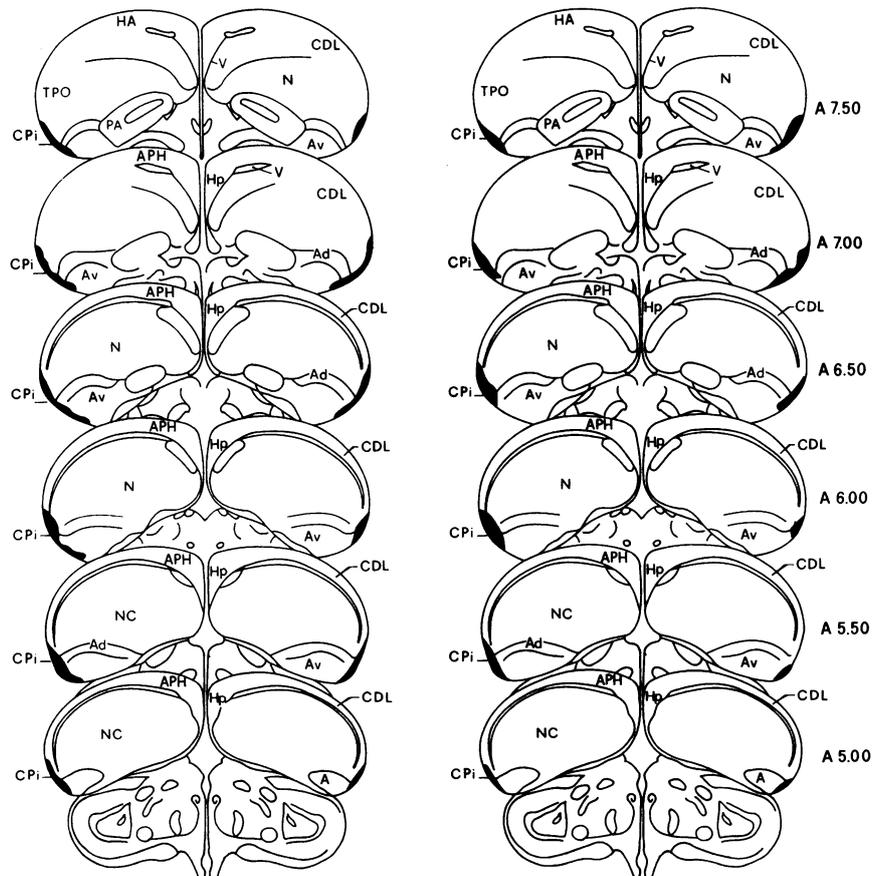
FIG. 1. CPi-ablation plots (filled areas) for two pigeons not used in the homing experiments. Drawings of the transverse sections and stereotaxic coordinates were derived from the atlas of Karten and Hodos (20). A, archistriatum; Ad, archistriatum, pars dorsalis; APH, area parahippocampalis; Av, archistriatum, pars ventralis; CDL, area corticoidea dorsolateralis; CPi, cortex piriformis; HA, hyperstriatum accessorium; Hp, hippocampus; N, neostriatum; NC, neostriatum caudale; PA, paleostriatum ovoidalis; TPO, area temporo-parieto-occipitalis; V, ventriculus.
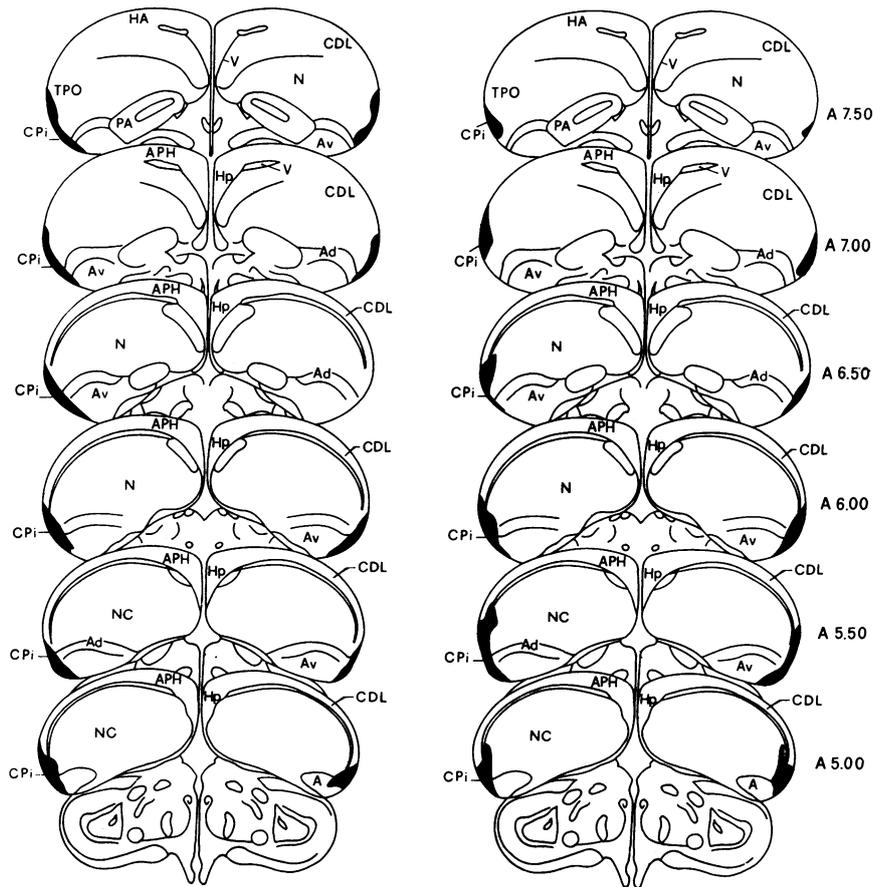
FIG. 2.   CPi-ablation plots (filled areas) for two pigeons that homed after test releases. Other explanations as in Fig. 1.

only 13 C and 10 E bearings were recorded. In both groups there was a significant clustering of bearings around the home direction ($P < 0.01$, Fig. 3 *A1–2*). The bearing distributions were not significantly different from each other ($P > 0.10$).

At the first unfamiliar site (Fig. 3 *B1–2*), C birds were homeward oriented ($P < 0.01$), whereas E birds were not (P

> 0.10). The difference between the two bearing sets does not reach significance ($0.05 < P < 0.10$). At the second unfamiliar site (Fig. 3 *C1–2*), C birds deflected strongly in a counterclockwise direction from the home bearing and the E birds deflected even more strongly. The two bearing sets do not show significant clustering around the home direction but
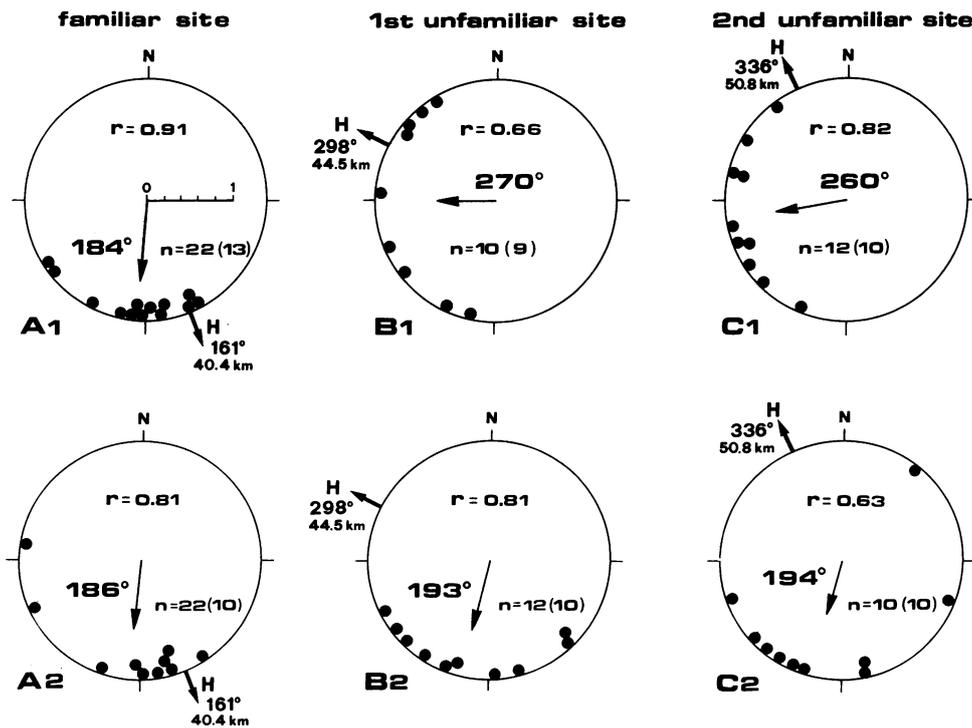
FIG. 3.   Diagrams showing the initial orientation in test releases from the familiar site (*A1*, *A2*) and from the unfamiliar sites (*B1*, *B2* and *C1*, *C2*). C birds are in the upper row and E birds are in the lower row. Each dot at the periphery of the circle indicates the bearing of one bird. The arrow outside the circle indicates home, whose direction and distance are given. The inner arrow indicates the mean vector, whose bearing and length (r) are given; n refers to the birds released (and bearings recorded).
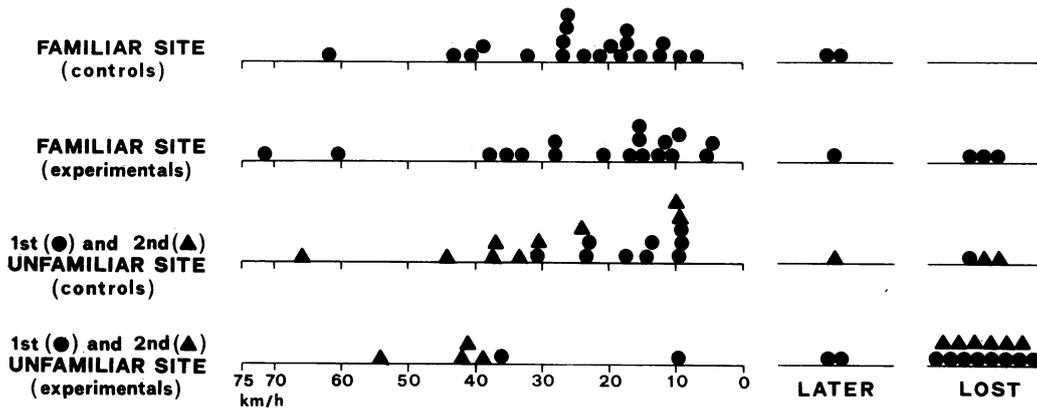
FIG. 4.   Homing performance in the test releases. Symbols of birds that homed during the day of release are arranged according to speed.

differ from each other ($P < 0.05$). When the bearings recorded at both unfamiliar sites are pooled by setting the home direction to $0°$, the bearing set of C birds shows a significant clustering around the home direction ($P < 0.01$), whereas that of E birds does not. The two sets are significantly different from each other ($P < 0.02$).

Homing performances are shown in Fig. 4. Twenty C birds of 22 and 18 E birds of 22 homed on the day of their release from the familiar site. The performance of E birds is marginally, but not significantly, inferior to that of C birds ($P > 0.10$). Three E birds were lost, whereas all of the C birds homed. In both releases from the unfamiliar sites, E birds performed more poorly than C birds, but the difference only reached significance in the first release ($P < 0.01$). By pooling the data of both releases, the difference becomes apparent ($P < 0.01$). In fact, 14 E birds of 22 were lost, whereas only 3 C birds of 22 did not home.

No differences in vanishing intervals were found.

Visceral Responses. On the first trial, 1 day before E pigeons underwent CPi ablation, all of the animals displayed a highly significant ($P < 0.001$) increase in heart rate following presentation of the olfactory stimulus. Six days after CPi ablation, one of four E birds did not show a significant increase in the heart rate, whereas the other three pigeons showed clear responses, though with a lower degree of significance ($P < 0.05$ to $P < 0.001$). The same phenomenon was observed in the C birds. In fact, in the second trial, one of six controls showed no significant increase in heart rate following olfactory stimulation, whereas in the other five pigeons less significant responses ($P < 0.02$ to $P < 0.01$) were observed.

DISCUSSION

The present results show that CPi-ablated pigeons are heavily impaired in initial orientation and homing ability when released from unfamiliar sites but not from familiar ones.

The CPi-ablated pigeons released from the unfamiliar sites had almost all homed previously from the familiar release site, so that their poor performance can hardly be attributed to aspecific disturbance and demotivation. Brain lesions *per se* do not produce failure to establish the home direction from unfamiliar sites, as shown by pigeons subjected to Wulst or hippocampal ablation (15, 16).

The initial orientation of C birds from familiar and unfamiliar sites shows a tendency to deflect toward SW. This can be attributed to the fact that the preferred compass direction of the pigeons from the Arnino loft is SW. However, in two of the three releases and in the pooled distribution, a significant clustering around the home direction was evident. The E birds took off in the same direction in all three releases. Our interpretation is that at the familiar site they were able to fix

the home bearing, whereas at the unfamiliar site they were unable to do this and simply flew in the training direction. This failure in navigation cannot be attributed to a disruption of the compass mechanism, as the birds were able to orient in the same direction in the presence of different, unfamiliar landscapes. Thus, the observed effect must be attributed to a disturbance in the map step of the navigational process.

Thus far, the same effect of CPi ablation on homing behavior—i.e., impairment in orientation and homing from unfamiliar but not from familiar sites—has been obtained only by experiments of olfactory deprivation (12–14). One of these experiments (13) is very similar to the present one in terms of experimental design, distance of the release sites from home, and results.

The percentage of CPi-ablated pigeons that did not home from the unfamiliar sites recorded by us (64%) is consistent with previous data (24) about the homing success of homing-experienced, anosmic pigeons released from unfamiliar sites. At 30 km from the loft, the percentage of birds lost is reported to be 52.5%, and at 150 km, 97.4%. The decrease in homing success with increasing distance is attributable to the lower chance of finding familiar landmarks allowing location of the loft.

The disruption of homing ability from unfamiliar sites found in CPi-ablated pigeons indicates that the CPi plays an important role in navigation. Since it receives projections from the olfactory bulb (19) and is probably involved in processing olfactory information, the effect of its ablation on a pigeon's navigational ability is consistent with the primary role of olfactory information in the homing mechanism.

The results obtained in the experiments in which heart rate changes were observed following presentation of an olfactory stimulus show that CPi does not necessarily play an important role in the mechanisms underlying visceral motor responses to olfactory stimulation. In fact, the responsiveness of CPi-ablated birds does not differ from that of controls tested at the same time. We can therefore hypothesize that the CPi does not function as a simple olfactory relay in which olfactory signals are processed independently of their behavioral significance but, rather, plays an important role in the specific mechanisms linking olfactory inputs to the behavioral orientation response.

It is interesting to note that in rats too the CPi is presumably involved in olfactory information processing. In fact, transection of the lateral olfactory tract (which carries inputs from the olfactory bulb to the CPi), on the one hand, results in reduced pyriform activity (evaluated with 2-deoxyglucose uptake; ref. 25) and, on the other hand, induces anosmic-like behavior in the rat (26). In addition, recent behavioral experiments suggest that the CPi in rats is necessary not only to perform olfactory discrimination tasks but also to build neural representations of olfactory cues (27).

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