The paratympanic organ (PO) in birds is a specialised sensory organ whose function is still unknown. G. Vitali, who first described the PO from observations of the behaviour of PO-lesioned pigeons, proposed that this organ was needed to maintain normal wing muscle function and called it the ‘organ of flight’. This interpretation has since been disputed. To solve this controversy and to test whether the PO is involved in flight and navigation, we performed release tests on homing pigeons subjected to bilateral destruction of this organ. No impairment of either flight or navigation was observed in the lesioned pigeons.

Key words: pigeon, Columba livia, paratympanic organ, lesion, homing.

Introduction

In birds, the paratympanic organ (PO) is a small sensory organ located in the medial wall of the tympanic cavity near the surface of the mucosa; its functions are yet to be specified. Since Vitali (1911) described the PO in the domestic sparrow (Passer domesticus), the morphology of this organ has been the subject of several structural and ultrastructural studies. The PO is a small, tapering vesicle containing a mucus-like fluid (Vitali, 1912, 1913). The medial wall of the organ is composed of a sensory epithelium which appears similar to that of the lateral line organs and the statokinetic apparatus of fish and amphibians. The epithelium consists of supporting cells and type II hair cells (Jørgensen, 1984; Giannessi and Pera, 1986; Giannessi and Ruffoli, 1996a), the latter supplied by both afferent and efferent fibres running in the facial nerve (Vitali, 1912, 1913). The medial wall of the organ is composed of a sensory epithelium which appears similar to that of the lateral line organs and the statokinetic apparatus of fish and amphibians. The structural complexity of the PO suggests that it has important functions; moreover, as the organ is typical of birds, previous authors have generally hypothesised functions related to flight; however, conclusive evidence for this is still missing. Indeed, the only behavioural and functional studies to date were carried out at the beginning of this century and yielded contradictory results. Vitali (1921), on the basis of the results of PO lesion experiments, proposed a functional mechanism by which the organ could detect atmospheric pressure changes and, at the same time, control the wing muscles. Vitali bilaterally removed the PO from pigeons by using thermoncaterory or by scraping the mucosa using a small spoon and observed a reduced tone and progressive atrophy of the wing muscles. Thus, the birds became unable to lift their wings and showed a drastic impairment in flight performance. As a consequence of these experiments, the author called the PO ‘l’organo del volo’ (the organ of flight). Lesion experiments using the same techniques were later repeated on pigeons by Benjamins (1926) and did not produce any kind of postural deficit, but the author did not record systematic data on homing behaviour of the lesioned birds. Moreover, neither Vitali (1921) nor Benjamins (1926) described the consequences of the ablation of the PO on the tympanic membrane and the columella auris.

Since then, research interest has focused on morphological and cytological features of the PO and, despite speculation on the nature of the stimuli to which this organ might be sensitive (air pressure changes, infrasound) and on its use in different situations (navigation, migration, flight), difficulty in reaching and removing this organ in vivo has so far prevented scholars from repeating functional studies using lesioned birds.

Our work had several purposes; first, we aimed to develop a surgical technique for the removal of the PO without damaging other structures. Then, by performing homing releases with lesioned pigeons, we intended to solve the controversy surrounding the involvement of the PO in flight behaviour and to test a possible role of this organ in orientation.

Materials and methods

Pigeons

Twenty-five adult homing pigeons (Columba livia L.) with previous homing experience, housed in a loft in Arnino (Pisa, Italy), were used in the experiment. Some pigeons (N=13) were
subjected to bilateral lesion of the PO while the remaining birds (N = 12) were used as intact controls.

Surgery
The birds were deeply anaesthetised by injecting a 20% solution of chloralhydrate (dose 2 ml kg\(^{-1}\)). The head of the pigeon was placed on a wooden plane inclined at approximately 30\(^\circ\) to the horizontal, so as to allow a lateral view. The head was held still by a cloth, pinned to the wooden plane. A hole in the cloth left the ear region uncovered. Surgery was carried out with the aid of a stereoscopic microscope. To reach the tympanic cavity, we used the transmeatal approach in accordance with a surgical technique similar to that used in human tympanotomy (Portmann, 1975). First, we created a posterior flap by making a clockwise semi-circular incision (using a Rosen’s curved lancet), positioned approximately 3 mm from the tympanic sulcus. Then we disconnected the cutaneous flap from the underlying osseous tissue as far as the sulcus using a rounded blade scraper. After detaching the posterior part of the tympanum, we reached the tympanic cavity. Using this technique, we obtained a good view of the tympanic cavity, particularly of the columella and of the area in which the PO is situated (Vitali, 1912). We raised the osseous thin wall covering the PO using a Shambaugh’s hook angled at 45\(^\circ\); the PO was then removed using a cofosurgery aspirator. During this operation, the integrity of the columella was carefully maintained. Finally, we repositioned the tympanic flap, applying some Gelfoam wads into the external acoustic meatus, in order to keep the meatal flap flat and make it adhere to the bone surface. Otoscopical examinations carried out after 10 days showed complete anatomical recovery of the tympanic membrane.

Release tests
After the surgery, the birds were allowed at least 10 days to recover. During the first test, the birds were carried 2 km from the loft, and the operated and control pigeons were released in two separate groups to test for a possible difference in their flight ability. The PO-lesioned birds (PO-l) and the intact controls (C) were then subjected to three further releases, the first of which was carried out at a site within their familiar area (R1, La Costanza), while the second and the third tests (R2, Marinella; R3, Monteriggioni) were at sites totally unfamiliar to the birds (see Table 1 for details). The release tests were carried out in sunny conditions with no wind. The pigeons were released singly and observed using 10x40 binoculars until they vanished from the observer’s sight. With the aid of a stopwatch and a compass, vanishing bearings and vanishing times were recorded for each pigeon. Homing times were also recorded.

Statistics
From the vanishing bearings, the mean vector length and direction were calculated for each bird group. Bearing distributions for each group were tested for randomness using the Rayleigh test; the Watson \(U^2\)-test was used to test for differences between the bearing distributions of the control and operated birds (Batschelet, 1981). The Mann–Whitney \(U\)-test was used to compare the homing performance of control and lesioned pigeons (Siegel, 1956).

Histology
At the end of the experiments, all the homed operated pigeons were deeply anaesthetised with pentobarbital and perfused through the left ventricle with Bouin’s fluid. The middle and inner ear were dissected out and immersed in Bouin’s fluid for 24 h. They were then decalcified, paraffin-embedded, serial-cut and finally stained with haematoxylin and eosin.

Results

<table>
<thead>
<tr>
<th>Home direction (degrees)</th>
<th>Distance (km)</th>
<th>Date</th>
<th>Treatment</th>
<th>n (N)</th>
<th>( r )</th>
<th>( \alpha ) (degrees)</th>
<th>Homeward component</th>
<th>( U^2 )-test</th>
<th>Homing success</th>
<th>U-test</th>
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<tr>
<td>190 (R1)</td>
<td>18</td>
<td>23.11.94</td>
<td>C</td>
<td>10 (10)</td>
<td>0.87***</td>
<td>208</td>
<td>0.83</td>
<td>NS</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PO-l</td>
<td>10 (10)</td>
<td>0.73**</td>
<td>232</td>
<td>0.54</td>
<td></td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>152 (R2)</td>
<td>50.3</td>
<td>6.12.94</td>
<td>C</td>
<td>7 (9)</td>
<td>0.67*</td>
<td>122</td>
<td>0.58</td>
<td>*</td>
<td>8</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>PO-l</td>
<td>8 (10)</td>
<td>0.61*</td>
<td>203</td>
<td>0.38</td>
<td></td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>293 (R3)</td>
<td>79</td>
<td>1.2.95</td>
<td>C</td>
<td>10 (10)</td>
<td>0.94***</td>
<td>302</td>
<td>0.93</td>
<td>NS</td>
<td>9</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>PO-l</td>
<td>9 (10)</td>
<td>0.96***</td>
<td>312</td>
<td>0.90</td>
<td></td>
<td>8</td>
<td>2</td>
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R1, familiar site; R2 and R3, unfamiliar sites; n, number of vanishing bearings recorded; N, number of pigeons released; \( r \), mean vector length; \( \alpha \), mean vector direction; \( U^2 \)-test, comparison between distributions C versus PO-l (Watson \( U^2 \)-test); homing success (in number of birds); \( U \)-test, comparison between homing performances, C versus PO-l (Mann–Whitney \( U \)-test); the levels of significance for the Rayleigh test for randomness are indicated in column \( r \); *P<0.05; **P<0.01; ***P<0.001; NS, not significant.

C, controls; PO-l, paratympanic-organ-lesioned pigeons.
indistinguishable from the control group and no movement impairment was observed in the lesioned group. The results concerning the initial orientation in single releases from the three further release sites are shown in Table 1.

Two control birds and two operated birds for site R2 and one operated bird for site R3 landed soon after being released. The vanishing times of the two groups of remaining pigeons were significantly different (Mann–Whitney $U$-test, $P<0.05$) in only one test (R3), the operated birds being faster than the control ones (median: C, 3 min 23 s; PO-l, 2 min 39 s).

The bearing distributions of both intact and operated birds were significantly different from random in all the test releases (see Table 1; Fig. 1). Operated birds differed from control birds in their initial orientation in one (R2) out of the three tests (Watson $U^2$-test: $P<0.05$, see also Table 1 and Fig. 1); the lesioned birds showed a strong tendency to fly southwest which, for the pigeons housed in Arnino, corresponds to the preferred compass direction (PCD) (Wallraff, 1978; Ioalè, 1995, 1996) (see Discussion for details). Both groups of pigeons showed an equally proficient homing success in all releases (Mann–Whitney $U$-test, $P<0.1$; see Table 1). Therefore, no difference was observed between the navigational abilities of the lesioned pigeons compared with those of the control ones.

**Histology**

Fig. 2A shows the osseous crista of the medial wall of the tympanic cavity containing the PO. The PO is surrounded by connective tissue and is covered by two osseous laminae which are slightly separated (arrowheads, Fig. 2A). Thus, a thin layer of connective tissue separates the PO from the lumen of the tympanic cavity. After the surgical removal of the PO (Fig. 2B), the osseous laminae covering the organ had been removed and a layer of connective tissue occupied the region where the PO was situated. The operation clearly did not damage the structures located beneath the PO.

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**Fig. 1.** Initial orientation of control and paratympanic organ (PO)-lesioned pigeons for the three different release sites (see Table 1 for details). H indicates the direction of the home loft. The mean vector directions and lengths are shown by the arrows for control (open arrowhead) and PO-lesioned (filled arrowhead) birds. A scale bar for vector length is shown in the left panel. N, magnetic north.

**Fig. 2.** (A) Transverse section through the tympanic cavity of a non-operated pigeon. The paratympanic organ (PO, large arrow) is located near the free surface of the mucosa (small arrows) and is partly covered by two osseous laminae (arrowheads). Asterisk, tympanic cavity. Scale bar, 300 $\mu$m. (B) Transverse section through the tympanic cavity of an operated pigeon. The region where the PO was located is now occupied by connective tissue (arrow; compare with A). The arrowhead points to the zone of breakage of one of the osseous laminae previously covering the PO. The deep structures appear to be intact with respect to non-lesioned pigeons (see A). Scale bar, 300 $\mu$m.
phenomenon has frequently been described in the literature towards their preferred compass direction (PCD; south or deviation might be explained as an increased tendency to fly experimental birds showed a deviation towards southwest. This Table 1; Fig. 1). In this test, the initial orientation of the unfamiliar site, the initial orientation of the lesioned birds differed significantly from that of the intact pigeons (R2, see Table 1; Fig. 1). Note that the PCD phenomenon is subject to greater variability than other factors affecting initial orientation (e.g. clock-shift treatment, olfactory deprivation) (see also Papi, 1995).

On the basis of the results achieved under our experimental conditions (clear sky, light or no wind, release sites up to 79 km from home), there is no evidence to suggest an involvement of the PO in the pigeon’s navigational system. However, further investigations are needed to draw definitive conclusions and to exclude the participation of the PO in the homing processes.

Our experiments do not invalidate several of the hypotheses suggested for the function of the PO (as an altimeter, barometer, infrasound detector). In addition, we suggest that the PO may be involved in a reflex allowing fast opening of the pharyngeal orifice of the Eustachian tube during rapid changes in altitude during flight, in order to balance air pressures between the middle ear and the external auditory meatus.

In conclusion, the functions of the PO remain unknown and further studies are needed to test all the hypotheses proposed to date. However, we have developed a technique for the production of a specific and reproducible lesion of the PO. The reliability of our surgical method is verified by the histological results and by the absence of any secondary effects resulting from lesion of the adjacent structures of the middle and inner ear. We believe that this method could be a useful tool for future research on the PO.

**References**


