J. exp. Biol. 164, 171–187 (1992)
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INTRA-NASAL ZINC SULPHATE IRRIGATION IN PIGEONS: EFFECTS ON OLFACTORY CAPABILITIES AND HOMING

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Accepted 14 November 1991

Summary

Depriving homing pigeons of olfactory information by applying a local anaesthetic, gingicain, to their olfactory epithelium results in systemic effects. Furthermore, anosmia persists reliably for only an hour. In contrast, intra-nasal irrigation with 18 % zinc sulphate solution results in anosmia that persists for at least 5 days. No systemic effects of zinc sulphate on anosmic pigeons could be detected while monitoring their olfactory capabilities using the orienting response. To compare the orientation and homing of definitely anosmic, zinc-sulphate-treated pigeons with those of controls, I made releases from two different distances (9–24 km, 63–70 km). At shorter distances, pigeons treated with zinc sulphate showed no directional preference in their vanishing bearings, whereas both groups of control birds (sham-treated controls and untreated super controls) were well oriented. At the longer distances, the situation was more complex: pigeons treated with zinc sulphate were oriented but the chosen direction did not correspond with the home direction. The control groups did not show any directional preference. In all cases, fewer anosmic pigeons homed and they did so more slowly than did the controls.

Introduction

Over the last 15 years the hypothesis of olfactory navigation has been the main focus of attention in the study of pigeon orientation (reviewed by Schmidt-Koenig, 1987; Waldvogel, 1989; Papi, 1990; Wallraff, 1990). Local anaesthesia (with gingicain or xylocain) of the nasal mucosa (Schmidt-Koenig and Phillips, 1978) has become a common method of depriving pigeons of olfactory information. This is an easy procedure. But the first animals regain their olfactory capacities after 1 h (Wallraff, 1988; Schlund, 1990, 1991*a*). In addition, recent investigations showed that local anaesthesia causes systemic effects (Schlund, 1990, 1991*a*) that are likely to reduce, indirectly, the navigational performance of pigeons (Wenzel and Rausch, 1977; Wenzel, 1982, 1983).

In this study I shall introduce intra-nasal irrigation with zinc sulphate ($ZnSO_4$) as a new method for olfactory deprivation in pigeons: it acts as an alternative to the

Key words: pigeon, zinc sulphate, anosmia, olfactory navigation, homing, *Columba livia*.

anaesthetization of the nasal mucosae but does not forfeit the advantages of that technique.

Intra-nasal treatment with $ZnSO_4$ solution in appropriate concentrations alters or destroys the olfactory mucosa. This has so far been tested in rats, dogs, apes, frogs, rabbits, mice and fish. The olfactory mucosa regenerates with time (Smith, 1938; Hunnicutt, 1939; Schultz, 1941; Smith, 1951; Mulvaney and Heist, 1971; Margolis *et al.* 1974; Matulionis, 1975, 1976; Harding *et al.* 1978; Cancalon, 1982). However, the duration of the regeneration period of the treated tissue varies considerably between investigations. It ranges from a few days in catfish (Cancalon, 1982) to several months in laboratory mice (Matulionis, 1975). Differences in regeneration time parallel the degree of tissue destruction. In this treatment, the duration of the application to the tissue is more important than the concentration of the $ZnSO_4$ solution (Cancalon, 1982).

Most investigations confirmed that the effect of $ZnSO_4$ is confined to the olfactory mucosa. However, Margolis *et al.* (1974) and Harding *et al.* (1978) discovered alterations and weight loss of the bulbus olfactorius in laboratory mice. These effects could still be detected 1 year after the $ZnSO_4$ treatment. Alberts and Galef (1971) undertook behavioural tests in rats in order to check whether the destruction of the olfactory mucosa resulted in anosmia. It was shown that the animals were unable to find hidden food pellets even 5–7 days after the application of $ZnSO_4$. Application of $ZnSO_4$ has also been found to be effective in hamsters, mice and sea turtles (reviewed by Alberts, 1974).

Thus, intra-nasal $ZnSO_4$ treatment has the following advantages. (1) Anosmia is rapidly and simply accomplished. (2) Animals regain their olfactory capacities after a few days. (3) Respiration is not hampered. (4) No systemic effects are known.

In the current study I tested whether the above results also apply to pigeons. I investigated the following questions. (1) What $ZnSO_4$ concentration most reliably disables the olfactory perception of pigeons? (2) How long does anosmia persist? (3) Does the $ZnSO_4$ treatment result in systemic impairments comparable to those observed after local anaesthesia of the nasal mucosa? (4) Does $ZnSO_4$ treatment alter homing ability in pigeons?

To test the first three questions I monitored spontaneous changes in heart beat frequency (orienting response). This method has proved to be suitable for testing the perceptual capabilities of pigeons in response to different environmental stimuli (Wenzel, 1967; Cohen and MacDonald, 1971; Quentmeier, 1986, 1989; Schlund, 1990). I tested the fourth question by means of releases from two different distances. I compared initial orientation and homing success of $ZnSO_4$-treated pigeons with those of untreated controls.

Materials and methods

Experimental birds

Pigeons (*Columba livia*), aged between 1 and 3 years, were housed at our loft

near Tübingen (Germany). In the beginning, none of the animals had experience of the laboratory experiment. They were used in olfactory experiments several times but, to avoid habituation, the same pigeon was never tested twice within a week. Pigeons used for releases had participated in single and flock releases from different distances (up to 60 km) in all cardinal directions from the loft. All birds were unfamiliar with the release sites.

Laboratory experiments

Test apparatus and techniques for the olfactory tests

By monitoring spontaneous changes in heart beat frequency, I tested the olfactory capabilities of the pigeons in response to different odorous stimuli. The odorous stimuli were air saturated with either lavender oil or rose oil. These two substances do not stimulate the nervus trigeminus and therefore have been widely used in olfactory tests, even in human medicine (Boenninghaus, 1986; Rentzsch, 1988). Odorous stimuli were applied for 4 s. The difference between heart beat frequencies at 8 s pre-stimulus and 8 s post-stimulus was taken as the criterion of response to the stimulus [cardiac response (Δhb/8 s)]. (For further description of the experimental apparatus, see Schlund 1990, 1991*b*.)

Definition of smelling

A prerequesite for the test of odour detection was a quick and dependable classification of the pigeons into 'smelling' and 'non-smelling' categories. To accomplish this I compared cardiac responses before and after treatment with a local anaesthetic (gingicain), to act as the control situation, *versus* odorous stimuli (lavender oil and rose oil). The 99.9 % confidence intervals of the median for the cardiac responses proved to be good criteria for such a classification (Fig. 1). 'Smelling' pigeons were those that showed a cardiac response of more than 3 beats in the 8 s interval after odour stimulus. 'Non-smelling' pigeons were those that showed a cardiac response of less than 2 beats in the 8 s interval after stimulus. Cardiac responses of exactly 2 or 3 beats in the 8 s post-stimulus interval were rated as indistinct.

Optimising the treatment with $ZnSO_4$

The following treatments, each based on the results from the previous one, were tested (percentages of $ZnSO_4$ solutions refer to $ZnSO_4 \cdot 7H_2O$).

(1) *1 % $ZnSO_4$ solution*. 1 g of $ZnSO_4 \cdot 7H_2O$ (287.45 g mol^{-1}) dissolved in 99 g of distilled water. Squirting 0.1 ml into each nostril with a one-way syringe.

(2) *6 % $ZnSO_4$ solution*. 6 g of $ZnSO_4 \cdot 7H_2O$ in 94 g of distilled water. Spraying approximately 0.2 ml into each nostril with a pump atomizer.

(3) *17.4 % $ZnSO_4$ solution*. 17.4 g of $ZnSO_4 \cdot 7H_2O$ in 82.6 g of 1 % xylocain solution (local anaesthetic; Astra Chemicals GmbH). Spraying approximately 0.2 ml through the choanes into the nasal cavities with a pump atomizer. (The idea for this concentration came from Bob Madden, USA, who had done some work on pigeons treated with $ZnSO_4$.)

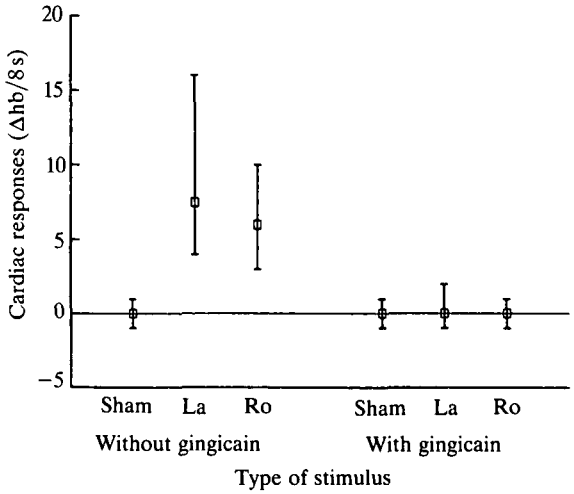

Fig. 1. The median and the 99.9 % confidence interval of the median of cardiac responses (Δhb/8 s, see Materials and methods) to a sham stimulus and to odorous stimuli (La=lavender oil and Ro=rose oil) before and after treatment with gingicain (N=30).

(4) *17.4 % $ZnSO_4$ solution.* 17.4 g of $ZnSO_4 \cdot 7H_2O$ in 82.6 g of distilled water. Spraying approximately 0.2 ml through the choanes with a pump atomizer.

(5) *17.4 % $ZnSO_4$ solution.* 17.4 g of $ZnSO_4 \cdot 7H_2O$ in 82.6 g of 8 % ethanol. Spraying approximately 0.2 ml through the choanes with a pump atomizer.

(6) *18 % $ZnSO_4$ solution.* 17.9 g of $ZnSO_4 \cdot 7H_2O$, 0.1 g of Myrj (emulsifier; trade name) and 35 g of Frigen 12 (propellant CCl_2F_2; trade name) in 25 % ethanol. Spraying approximately 0.2–0.3 ml through the choanes into the nasal cavities with an aerosol can.

(7) *18 % $ZnSO_4$ solution.* 11.2 g of $ZnSO_4 \cdot 1H_2O$ (179.45 g mol^{-1}), 0.1 g of Tagat 02 (emulsifier; trade name) and 25 g of Frigen 12 in 63.7 g of distilled water. Spraying approximately 0.2–0.3 ml through the choanes with an aerosol can.

Treatment 7 proved to be successful and was therefore used in all following experiments.

Comparison of cardiac responses resulting from different manipulations of the olfactory mucosae

Odour detection by pigeons, whose mucosae had been treated in different ways, was tested in response to lavender oil. The cardiac responses among the following groups were compared: (a) anaesthesia with gingicain (for reference), (b) treatment 7 with $ZnSO_4$ [experimentals ($ZnSO_4$-pigeons)], (c) sham-treated with C-$ZnSO_4$ [corresponding to treatment 7 without $ZnSO_4$; controls (C-$ZnSO_4$-pigeons)], (d) no treatment [super controls (SC)]. Groups b–d corresponded to the three groups used in releases.

Period of recovery from treatment with $ZnSO_4$

Olfactory perception in 16 pigeons was tested the day following the treatment and for each consecutive day until all the pigeons had regained their sense of smell.

Systemic impairments after $ZnSO_4$ treatment

Since local anaesthesia with gingicain results in systemic impairments (Schlund, 1990), I tested whether the $ZnSO_4$ treatment produced similar effects. I compared the heart beat frequencies and cardiac responses to optic and acoustic stimuli of $ZnSO_4$-treated pigeons with those of gingicain-treated pigeons and untreated super controls. For more details, refer to Schlund (1990).

Homing experiments

Release groups

Pigeons were subdivided into three release groups, which were as uniform as possible in age, experience and number. (1) Experimentals ($ZnSO_4$-pigeons); pigeons were treated on two successive days with $ZnSO_4$ (treatment 7) to maximise the number of anosmic birds. On the following day, the olfactory capabilities of each pigeon were tested. Only birds that proved to be anosmic were used for releases. Releases were carried out within 5 days of $ZnSO_4$ irrigation. Olfactory perception in $ZnSO_4$-pigeons which arrived at the loft after release was tested immediately (on average there was one smelling $ZnSO_4$-pigeon per release). Only the data for birds still found to be anosmic were considered for statistical analysis. (2) Controls (C-$ZnSO_4$-pigeons); controls were sprayed with C-$ZnSO_4$ on two successive days. (3) Super controls (SC); completely untreated pigeons.

Test sites and release procedure

Four sites between 9 and 24 km (distance I) and four sites between 63 and 70 km (distance II) from the loft and located as symmetrically as possible around it were used for releases. The pigeons were released singly, alternating experimental birds with controls and super controls. They were watched using 7×50 binoculars until they vanished from sight. The vanishing bearings were recorded to the nearest 1°. All releases were performed under sunny conditions in August, September and October in 1989 and 1990.

Data analysis

Linear statistics

Data were tested for normality (Lilliefors; Lorenz, 1988) and treated by either Kolmogorov–Smirnov or Student's *t*-test, accordingly.

Homing performances were compared with the Mann–Whitney *U*-test after standardisation of homing speed on the median of super controls (for standardisation, the homing speed from each pigeon in every group was divided by the median of the super controls. This was performed for each release separately

before summarising the data per distance and per group) (Siegel, 1956). Tests were run on a PC with the help of SAS (1987).

Circular statistics

For circular statistics, only vanishing bearings were considered. For each sample and release distance, I calculated the mean vector length, **a**, and direction, α (compass vector=mean direction of vanishing bearings with respect to north). Each sample was tested for directional preferences with the Rayleigh test (Batschelet, 1981). For significant samples, the 95 % confidence interval of the mean direction was used to test whether the mean direction differed from that of home ('Bootstrap' with 500 replica; Cabrera *et al.* 1991). Vanishing bearings of different samples were compared by means of first-order statistics (Watson U^2-test) and second-order statistics (Hotelling test).

Results

Laboratory experiments

Optimising the treatment with $ZnSO_4$

Fig. 2 shows the outcome of different applications with varying concentrations of $ZnSO_4$. The definitions for the various categories are given in Materials and

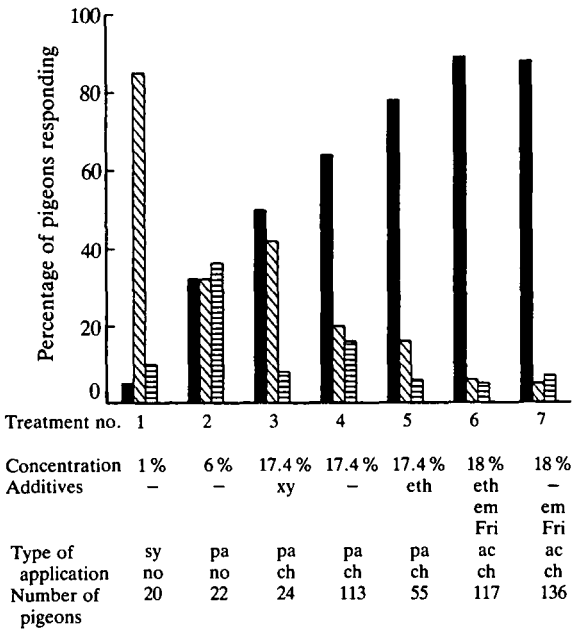

Fig. 2. Optimisation of the treatment with $ZnSO_4$; the percentage of the pigeons responding to the stimuli is shown. ■, non-smelling; ▨, smelling; ▤, indistinct. xy, xylocain; eth, ethanol; em, emulsifier; Fri, Frigen; sy, syringe; pa, pump atomizer; ac, aerosol can; no, nostrils; ch, choanes.

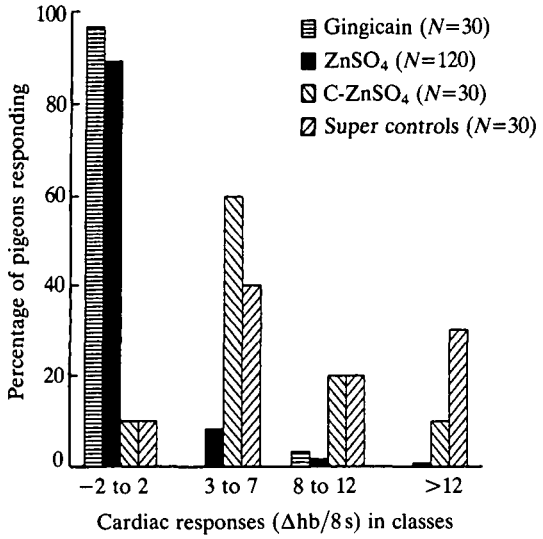

Fig. 3. Cardiac responses (Δhb/8s, see Materials and methods) of pigeons with differently manipulated olfactory mucosae in response to an odorous stimulus (lavender oil); cardiac responses are divided into four classes: −2 to 2, 3 to 7, 8 to 12, more than 12. For further details, see text.

methods. Based on these results, treatment number 7 was considered to achieve anosmia most reliably with the fewest side effects. This procedure was thus used in the homing experiments.

Comparison of cardiac responses to different manipulations of the olfactory mucosae

The responses of pigeons to the four treatments are given in Fig. 3. Comparisons among groups revealed significant differences between the two groups of controls and the two experimental groups treated either with $ZnSO_4$ or with gingicain (Kolmogorov–Smirnov test: $\chi^2 \geq 40.00$, d.f.=2, $P<0.001$). No difference was observed either between the two controls or between the two experimental groups (Kolmogorov–Smirnov test: $\chi^2<2.50$, d.f.=2; $P>0.05$).

Period of recovery from treatment with $ZnSO_4$

The first of the 16 pigeons recovered from the anosmia caused by $ZnSO_4$ on the sixth day. After 20 days, all the pigeons responded to the olfactory stimulus again (Fig. 4).

Systemic impairments after $ZnSO_4$ treatment

The heart rate (beats min^{-1}) of the $ZnSO_4$-pigeons was not different from the heart rate of the super controls (mean, standard deviation and sample size. $ZnSO_4$: mean=168±26.1, N=120; SC: mean=167±20.7, N=30; t-test: t=0.34, d.f.=148, $P>0.05$). In contrast, pigeons to which gingicain was administered

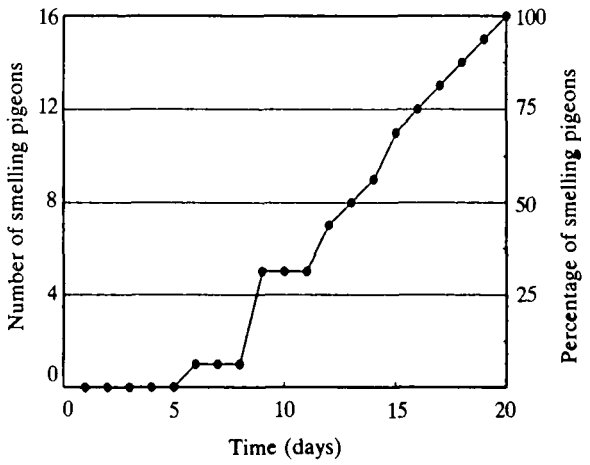

Fig. 4. Period of recovery from treatment with $ZnSO_4$. For further details, see text.

showed a reduction of the heart rate to 147±24.7 beats min^{-1} (N=30). Thus, the gingicain-treated group differed significantly from the super controls (t-test: t=2.87, d.f.=28, P<0.01) as well as from the $ZnSO_4$-pigeons (t-test: t=3.93, d.f.=148, P<0.001).

Changes in the heart rate of the $ZnSO_4$-pigeons did not differ from those of the super controls in response to either acoustic or visual stimuli [$ZnSO_4$: N=18, acoustic stimuli, mean (per 8 s)=7.1±4.0, visual stimuli, mean (per 8 s)=8.4±4.5; SC: N=15, acoustic stimuli, mean (per 8 s)=8.9±4.9, visual stimuli, mean (per 8 s)=9.8±7.5; t-test: t≤1.20, d.f.=31, P>0.05]. The pigeons to which gingicain (N=15) had been administered reacted far less to the visual stimulus [mean (per 8 s)=5.5±3.5] than did the super controls (t=2.85, d.f.=28, P<0.01) or the $ZnSO_4$-pigeons (t=2.07, d.f.=31, P<0.05). The response to the acoustic stimulus did not differ among pigeons treated with gingicain [mean (per 8 s)=6.3±5.3] or with $ZnSO_4$ (t=0.47, d.f.=31, P>0.05). Though not significant at the 5 % level, there was also a tendency for the gingicain-treated group to respond less to the acoustic stimulus than did the super control group (t=2.00, d.f.=28, 0.1>P>0.05).

Homing experiments

Releases from distance I

Table 1 summarises the principal data of the single releases. If pooled with respect to home, both groups of controls showed directional preference according to the Rayleigh test. The 95 % confidence interval of the direction of the mean vector included the home direction. The distribution of the pooled vanishing bearings of pigeons treated with $ZnSO_4$ was not different from random (Fig. 5).

In addition, if pooled with respect to the mean of super controls, the C-$ZnSO_4$-pigeons showed a directional preference, whereas the vanishing direc-

Table 1. *Initial orientation of pigeons in single releases from distance I*

Site	β	km	gr	α	a	n_a	n_{VR}	km h^{-1}	n_h
Ler	140°	15.4	$ZnSO_4$	47°	0.31	11	8	4.1	6
			C-$ZnSO_4$	153°	0.57*	12	10	22.0	11
			SC	176°	0.08	12	10	22.7	12
Fro	45°	15.8	$ZnSO_4$	355°	0.32	9	8	0.7	7
			C-$ZnSO_4$	38°	0.92***	9	9	39.5	9
			SC	19°	0.39	9	8	37.9	8
Pli	221°	23.4	$ZnSO_4$	359°	0.31	11	11	0.4	6
			C-$ZnSO_4$	229°	0.39	12	9	8.9	11
			SC	261°	0.75***	12	11	25.8	12
Duß	342°	9.2	$ZnSO_4$	153°	0.62*	12	8	8.8	9
			C-$ZnSO_4$	75°	0.36	12	8	28.3	12
			SC	321°	0.31	12	12	27.7	12

Site, release site (Ler=Lerchenberg, Fro=Frommenhausen, Pli=Plieningen, Duß=Dußlingen); β, home direction; km, distance of release site from home; gr, release groups ($ZnSO_4$=experimentals, C-$ZnSO_4$=controls, SC=super controls); α, **a**, direction and length of mean vector (north=0°); levels of significance under the Rayleigh test (* $P<0.05$, *** $P<0.005$); n_a, number of pigeons released; n_{VR}, number of vanishing bearings; km h^{-1}, median of homing speed; n_h, number of pigeons homed within 3 days.

tions of $ZnSO_4$-pigeons were randomly distributed (Fig. 6). The two groups of controls were indistinguishable in comparisons using both first- and second-order statistics (first-order: Watson U^2-test: $U^2 \leq 0.105$, $P>0.05$; second-order: Hotelling test: $T^2 \leq 0.58$, $F\,2,5$, $P>0.05$). First-order statistics assigned a difference between the $ZnSO_4$-pigeons and both groups of controls (Watson U^2-test: SC – $ZnSO_4$: $U^2=0.281$, $P<0.01$; C-$ZnSO_4$ – $ZnSO_4$: $U^2=0.411$, $P<0.001$). Second-order statistics depicted a significant difference only between C-$ZnSO_4$-pigeons and $ZnSO_4$-pigeons (Hotelling test: $T^2=31.68$, $F\,2,5$, $P<0.01$) but not between super controls and $ZnSO_4$-pigeons (Hotelling test: $T^2=6.83$, $F\,2,5$, $P>0.05$).

The homing speed of $ZnSO_4$-pigeons was slower than the speed of either group of controls. This was true for the single releases (Table 1) as well as for the combined releases pooled with respect to the median of super controls, as shown in Fig. 7 (Mann–Whitney U-test: $z \geq 5.35$, $P<0.001$). There was no difference between the two control groups (Mann–Whitney U-test: $z=0.42$, $P>0.05$).

Releases from distance II

Table 2 summarises the principal data of the single releases. If pooled with respect to home, neither group of controls showed a directional preference according to the Rayleigh test. The distribution of the pooled vanishing bearings of the $ZnSO_4$-pigeons, however, was different from random (Fig. 8). However, the 95 % confidence interval of the direction of the mean vector did not include the home direction. If pooled with respect to the mean of super controls, the

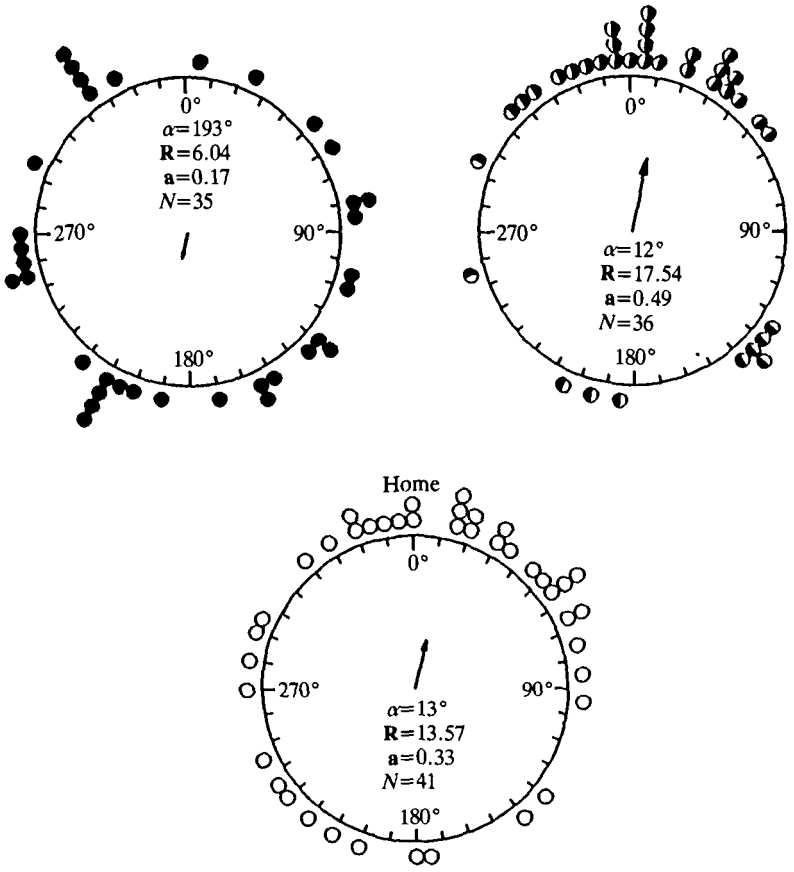

Fig. 5. Initial orientation of $ZnSO_4$-pigeons (●), C-$ZnSO_4$-pigeons (◐) and super controls (○) summarised with respect to home (=0°). Releases from distance 1. α, **R**, direction and length of the mean vector; **a**, length of the mean vector with respect to 1, N=sample size.

C-$ZnSO_4$-pigeons showed a directional preference, whereas the vanishing directions of $ZnSO_4$-pigeons were randomly distributed (Fig. 9). The two groups of controls were indistinguishable in both comparisons using both first-order and second-order statistics (first-order: Watson U^2-test: $U^2 \leq 0.058$, $P>0.05$; second-order: Hotelling test: $T^2 \leq 1.00$, F 2,13, $P>0.05$). First-order statistics assigned a difference between the $ZnSO_4$-pigeons and super controls (Watson U^2-test: $U^2=0.206$, $P<0.05$) but not between $ZnSO_4$-pigeons and C-$ZnSO_4$-pigeons (Watson U^2-test: $U^2=0.108$, $P>0.05$). Second-order statistics depicted no significant difference either between C-$ZnSO_4$-pigeons and $ZnSO_4$-pigeons (Hotelling test: $T^2=2.95$, F 2,13, $P>0.05$) or between super controls and $ZnSO_4$-pigeons (Hotelling test: $T^2=6.86$, F 2,13, $P>0.05$). As already shown for releases from distance I, the homing speed of $ZnSO_4$-pigeons was slower than the homing speed of either group of controls (Fig. 10). Combined releases were pooled with respect

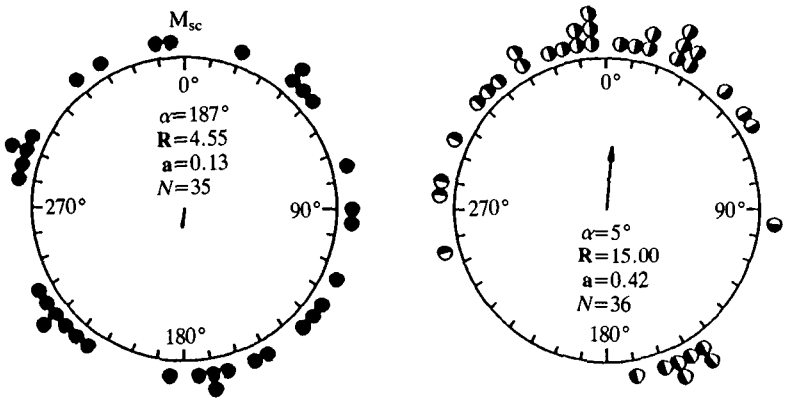

Fig. 6. Initial orientation of $ZnSO_4$-pigeons (●) and C-$ZnSO_4$-pigeons (◐) pooled with respect to the mean of the super controls (M_{SC}=0°). Releases from distance I. For more details, see Fig. 5.

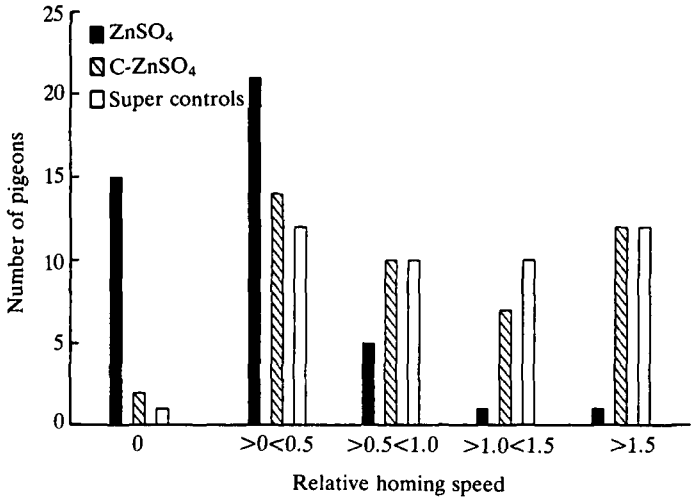

Fig. 7. Summary of relative homing speeds of the releases from distance I. For further details, see Materials and methods.

to the median of super controls (Mann–Whitney *U*-test: $z \geq 5.75$, $P<0.001$) and showed a significantly slower homing speed for $ZnSO_4$-pigeons. There was no difference between the two control groups (Mann–Whitney *U*-test: $z=1.13$, $P>0.05$).

Discussion

Laboratory experiments

In some vertebrates, intra-nasal irrigation with a 1–5% $ZnSO_4$ solution is

Table 2. *Initial orientation of samples of pigeons in single releases from distance II*

Site	β	km	gr	α	a	n_a	n_{VR}	km h^{-1}	n_h
Hoc	30°	67.2	$ZnSO_4$	22°	0.60	9	8	0.0	0
			C-$ZnSO_4$	9°	0.61*	10	8	30.2	8
			SC	35°	0.77***	10	8	35.0	9
			$ZnSO_4$	295°	0.60	7	6	0.0	2
			C-$ZnSO_4$	10°	0.88***	8	6	31.6	8
			SC	327°	0.35	8	7	36.9	7
Hap	199°	63.6	$ZnSO_4$	56°	0.11	11	7	26.2	4
			C-$ZnSO_4$	167°	0.24	12	10	25.1	11
			SC	181°	0.54	12	8	35.7	9
			$ZnSO_4$	51°	0.60	8	8	20.7	3
			C-$ZnSO_4$	82°	0.54	8	6	37.8	7
			SC	214°	0.33	9	5	42.0	8
Dur	129°	69.3	$ZnSO_4$	244°	0.08	12	10	0.0	1
			C-$ZnSO_4$	282°	0.58*	12	10	0.6	10
			SC	268°	0.61*	12	9	0.6	9
			$ZnSO_4$	288°	0.96***	8	6	0.0	0
			C-$ZnSO_4$	251°	0.40	10	8	14.8	9
			SC	221°	0.37	14	11	0.8	10
Tom	275°	69.3	$ZnSO_4$	93°	0.60*	10	9	0.0	9
			C-$ZnSO_4$	24°	0.36	11	9	0.0	5
			SC	30°	0.37	11	10	0.7	10
			$ZnSO_4$	195°	0.56	10	8	0.0	0
			C-$ZnSO_4$	217°	0.35	10	8	0.0	4
			SC	172°	0.33	10	9	0.8	6

This table shows two replicates for each release site.
Release sites: Hoc=Hochemmingen, Hap=Happenbach, Dur=Durmersheim, Tom=Tomerdingen.
For more details, see Table 1.

sufficient to achieve anosmia. In pigeons, however, 18% $ZnSO_4$ solution was required for total anosmia over a period of at least 5 consecutive days. These findings seem to agree with those of Bob Madden (unpublished data). The high concentration of 18% was necessary because of the short period of exposure when spraying the pressurised solution of $ZnSO_4$ into the nasal cavities. As Cancalon (1982) pointed out, the duration of irrigation is crucial for the degree of damage to the nasal mucosa. Prolonged irrigation, however, often results in degeneration of the nervus olfactorius and/or bulbus olfactorius. High concentrations of $ZnSO_4$ combined with a short exposure do less damage to these structures (Cancalon, 1982). Nevertheless, the treatment does not exclude effects on the nervus olfactorius and the bulbus olfactorius or adjacent parts of the brain, even though

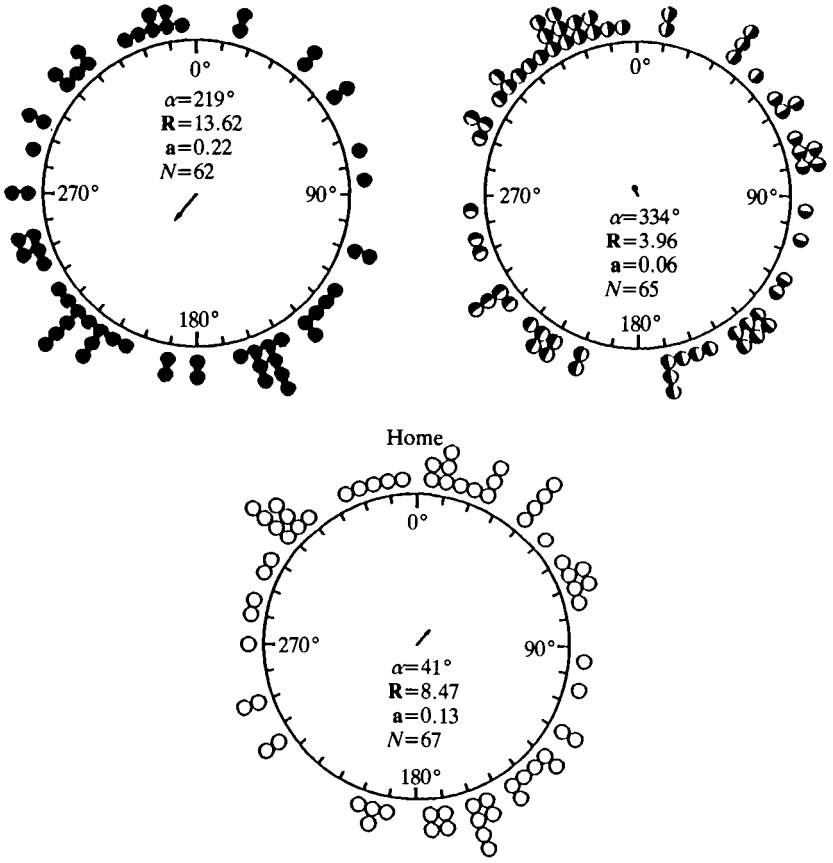

Fig. 8. Initial orientation of the $ZnSO_4$-pigeons (●), C-$ZnSO_4$-pigeons (◐) and super controls (○) summarised with respect to home (=0°). Releases from distance II. For more details, see Fig. 5.

systemic effects, such as those in pigeons treated with the local anaesthetic gingicain (Schlund, 1990), could not be detected. This has to be clarified through histological investigations. In contrast to my experiments, only behavioural tests have been carried out in mice, hamsters and rats to check the olfactory capabilities of the investigated animals. Since behavioural tests depend on the motivation of the animals, the use of cardiac response in this study was more reliable because heart rate acceleration is the result of a spontaneous reaction to stimuli.

Furthermore, the concentrations of odorous stimuli (air saturated with lavender or rose oil) used in this study were extremely high. Experiments with mice had shown that animals whose olfactory mucosae were damaged by up to 90% were still able to detect hidden food pellets (Harding *et al.* 1978). For pigeons not to smell the very intense stimuli (lavender oil and rose oil), their olfactory mucosae probably have to be completely destroyed.

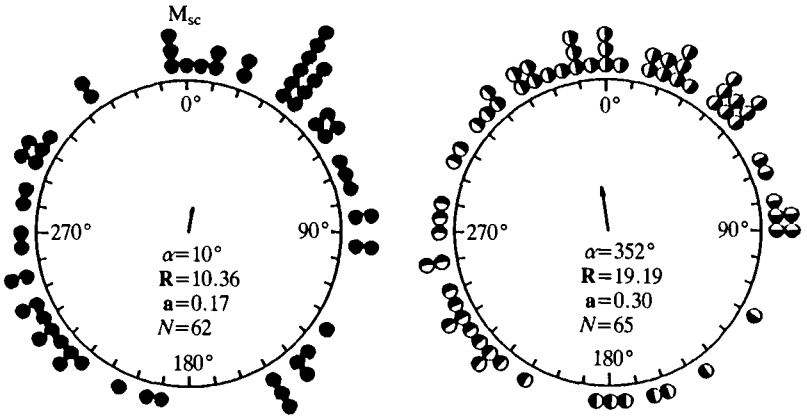

Fig. 9. Initial orientation of the $ZnSO_4$-pigeons (●) and the C-$ZnSO_4$-pigeons (◐) pooled with respect to the mean of super controls (M_{SC}=0°). Releases from distance II. For more details, see Fig. 5.

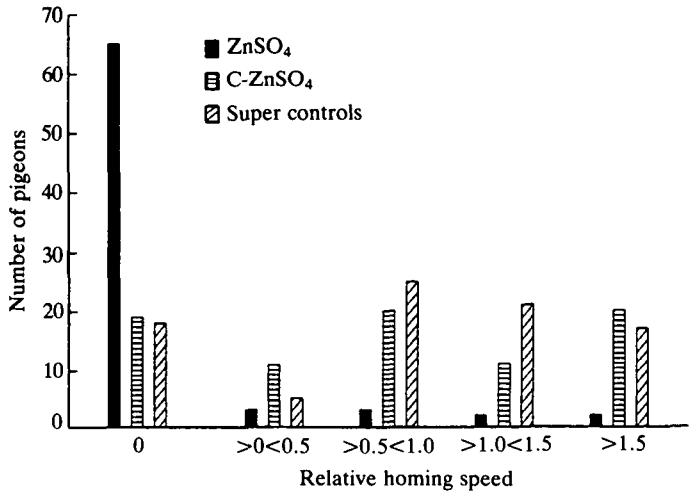

Fig. 10. Summary of relative homing speeds of the releases from distance II. For further details, see Fig. 7.

Homing experiments

At shorter distances (distance I) the vanishing bearings of anosmic $ZnSO_4$-pigeons were more scattered than the bearings of controls. In releases from distance II, the distribution of vanishing bearings did not differ between $ZnSO_4$-pigeons and both control groups. This comparison was hampered by the lack of directional preferences in the controls and super controls. Homing speed, however, was drastically reduced in $ZnSO_4$-pigeons of both series. These results

seem to support the hypothesis of olfactory navigation of Papi *et al.* (1972) and Wallraff (1974, 1980). However, it is too early to make a clear statement. Releases of $ZnSO_4$-pigeons from familiar sites showed that initial orientation did not differ between experimental and control groups (Schlund and Schmid, 1991). According to the current reasoning (Wallraff and Neumann, 1989), the anosmic pigeons should have been able to compensate for olfactory deprivation by using other cues for orientation at familiar sites (e.g. landmarks). It is startling that at releases from familiar sites the homing performances of $ZnSO_4$-pigeons and controls still differed dramatically (Schlund and Schmid, 1991).

These results indicate that treatment with $ZnSO_4$ has pronounced effects on the homing behaviour of pigeons. Whether this should be attributed to the deprivation of olfactory information or to side effects remains to be answered. As Schmidt-Koenig and Ganzhorn (1991) pointed out, the question of whether the olfactory system carries anything other than olfactory information is still not solved. Correspondence between the olfactory system and behavioural non-olfactory functions (e.g. learning, motivation and attentional behaviour), which probably have effects on navigation, has been clearly demonstrated (Wenzel, 1982). Treatment with $ZnSO_4$ causes weight loss of the bulbus olfactorius in mice (Harding *et al.* 1978), bulbectomy in rodents exerts a variety of behavioural and neuroendocrine effects (Jesberer and Richardson, 1986) and ablation of the mouse olfactory bulb modulates circadian rhythms (Possidente *et al.* 1990). If this holds true for pigeons, and if there are connections between the olfactory system and the circadian clock, manipulations to the olfactory system might change circadian rhythms. Circadian rhythms exert effects on migration and orientation in birds, e.g. *via* the time-compensated sun compass.

Little is known about the processing of navigational information in the brain of birds (reviewed by Semm and Beason, 1990*a*). Experiments to localise areas of navigational processing in the pigeon's brain have already been carried out (Bingman *et al.* 1989; Papi and Casini, 1990). However, large non-specific parts of the brain, in which the authors assumed the processing of olfactory inputs took place, were eliminated from consideration. Thus, these studies have proved insufficient to support clear statements. Semm and Beason (1990*b*) found electrophysiological responses to magnetic variations in the nervus ophthalmicus of the bobolink (*Dolichonyx oryzivorus*; the bobolink migrates over the equator using the earth's magnetic field for orientation). The ophthalmic nerve is the principal sensory nerve of the orbit and the nasal cavities (Bubién-Waluszewska, 1981). Impairments in the ophthalmic nerve would, therefore, be conceivable after intra-nasal irrigation with $ZnSO_4$ or other chemicals. This means that treatment with $ZnSO_4$ might interfere with magnetic orientation.

In view of these facts, it is of great importance to reveal details about brain area and the action of $ZnSO_4$ in the olfactory system and adjacent brain parts. Suitable methods for staining tissues sprayed with $ZnSO_4$ could perhaps make this possible. Once these areas have been identified, further specific elimination experiments may permit conclusions about navigational processing.

I wish to thank Professor Dr K. Schmidt-Koenig and Dr J. U. Ganzhorn for reading this manuscript and B. Madden for sharing his experience of how to apply $ZnSO_4$ in pigeons with me. Dr J. Burkhardt and F. Scharfe helped greatly in preparing the manuscript. M. Franck and H. Kaupp developed the appropriate zinc sulphate solutions in aerosol cans. This work was supported by the Deutsche Forschungsgesellschaft (SFB 307).

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