

RESEARCH ARTICLE

Back home at night or out until morning? Nycthemeral variations in homing of anosmic Cory's shearwaters in a diurnal colony

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SUMMARY

Olfactory cues have been shown to be important to homing petrels at night, but apparently those procellariiform species that also come back to the colony during the day are not impaired by smell deprivation. However, the nycthemeral distribution of homing, i.e. whether displaced birds released at night return to their burrow by night or during daylight, has never been investigated. To explore this question, we studied the homing behaviour of Cory's shearwater (*Calonectris borealis*) in the only known population where these birds are active at the colony both during the day and at night. Here, we compared the nocturnal *versus* diurnal homing schedule of birds treated with zinc sulphate (to induce a reversible but complete anosmia) with that of controls. Our results show that anosmic shearwaters were unable to home in the dark and were constrained to wait for the daylight to find their burrow again. Our results confirm that olfaction is the basic sensory input for homing by night even in a petrel species that is diurnally active at the colony.

Key words: anosmia, homing, olfaction, procellariiform seabirds, short-range orientation, zinc sulphate.

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INTRODUCTION

Short-range homing (i.e. the localization of the nest within the colony) of many petrel species has been shown to be driven by olfaction. Most procellariiform seabirds return to the colony exclusively by night (hereafter called 'nocturnal') despite their poorly developed night vision (Brooke, 1989; Martin and Brooke, 1991), suggesting that they may have evolved other ways to locate and identify their burrows in the dark. The characteristic musky scent of nocturnal petrels' burrows, together with their well-developed sense of smell, led scientists to hypothesize on the possible role of olfactory cues in the location and recognition of the nest (Bang, 1966; Bang, 1971; Healy and Guilford, 1990; Roper, 1999). Several studies investigated olfactory guidance to the colony and to the nest burrow employing different techniques, including smell deprivation induced by nerve section, plugging nostrils and zinc sulphate treatment (Benvenuti et al., 1993; Bonadonna and Bretagnolle, 2002; Bonadonna et al., 2001; Grubb, 1973; Grubb, 1974; Grubb, 1979). All of these studies reported poorer or null homing performances of anosmic birds compared with controls, emphasizing the crucial role of a functional sense of smell for homing at night. In addition, a number of behavioural studies carried out with Y-maze choice tests demonstrated the capacity of different species of procellariiforms for distinguishing between their own and a conspecific's burrow only by odour, as if the burrows had an individual olfactory signature (Bonadonna et al., 2003a; Bonadonna et al., 2003b; Bonadonna et al., 2004).

Petrel species that return to the colony by day (hereafter called 'diurnal') appear to be able to home from a short distance even if anosmic, showing homing performance no different to that of controls (Bonadonna and Bretagnolle, 2002). This suggests that only nocturnal species may have evolved the use of olfactory cues for homing. An interesting example is that of Cory's shearwater, a

nocturnal species that has been shown to be unable to home if anosmic when tested at a Mediterranean colony (Benvenuti et al., 1993), but apparently not influenced in its homing performance when tested in Selvagem Grande, an Atlantic island where this same species also has diurnal habits (Bonadonna and Bretagnolle, 2002). Petrel species considered diurnal are not generally constrained to home by day, but can be active at the colony both during the day and at night. Nocturnal petrels, by contrast, are constrained to home by night, mainly as an anti-predatory strategy (Mougeot and Bretagnolle, 2000; Warham, 1996).

However, the mentioned experiment in Selvagem Grande was conducted, in the majority of tested species, after inducing anosmia by plugging nostrils (Bonadonna and Bretagnolle, 2002). This method has been widely employed to induce smell deprivation both in petrels and homing pigeons (Grubb, 1974; Haftorn et al., 1988; Snyder and Cheney, 1975), but it is not completely reliable (as the authors point out). Bilateral occlusion of the nares clearly reduces olfactory sensitivity, but it does not make the bird anosmic: birds are obliged to breathe through the beak and odorous molecules may still reach the olfactory epithelium through the choanae (Wallraff, 1988). Consequently, we cannot be completely sure that successfully homing birds were actually unable to smell their burrows.

Furthermore, the procedure employed in all previous studies to check for differences in performance between anosmic and control birds might in fact only discriminate olfactory dependence in exclusively nocturnal homers. A daily check of experimental burrows during the morning is enough to assess the influence of olfactory impairment in nocturnal species because birds that are unable to home in the dark when anosmic are forced to wait until the treatment has worn off, so the count of days elapsed between release and homing is a good indicator of performance. The same

procedure may not be equally revealing when testing petrel species that can also home by day, because even if olfactory deprivation does indeed impair homing at night this may not show up in the daily check if birds can switch to non-olfactory homing at dawn. A detailed investigation of the nycthemeral distribution of homing, i.e. whether displaced birds released at night return to their burrow in the dark of the night or during the daylight, has never been carried out. An early experiment involving wedge-tailed shearwaters (*Puffinus pacificus*) deprived of smell by sectioning of the olfactory nerve illustrates this potential bias. Results showed that 25% of anosmic birds were able to home within 1 week of release (Shallenberger, 1975). This unusual finding for a nocturnal species was interpreted as a demonstration that olfactory cues are not essential for homing. However, the author also remarks that wedge-tailed shearwaters are not strictly nocturnal at the colony of Manana Island, where the experiment was carried out. So it remains possible that although anosmic birds were in fact unable to home at night, some instead may have homed during the day by relying on a sense other than olfaction.

The aim of our study is to investigate the nycthemeral distribution of homing behaviour of anosmic Cory's shearwater [*Calonectris borealis* (Cory 1881)], in the only known population where these birds are active at the colony both during the day and the night. In particular, we wanted to determine whether there is a difference in the nocturnal *versus* diurnal homing schedule of anosmic birds compared with controls, even if the general homing performance remains the same. We induced anosmia with zinc sulphate, a non-invasive chemical technique, to produce a temporary impairment of the olfactory mucosa (Benvenuti et al., 1992; Benvenuti et al., 1993; Bonadonna and Bretagnolle, 2002; Bonadonna et al., 2001; Guilford et al., 1998), and we released anosmic and control shearwaters at night to induce them to home to their burrows in the dark. We predicted that if diurnal Cory's shearwaters do not need olfaction and rely on other cues to home, as previously suggested (Bonadonna and Bretagnolle, 2002), then control and anosmic birds should not show any difference in the nycthemeral distribution of homing. However, if olfactory cues are essential for homing by night, only control birds will still be able to home in the dark while anosmic birds will not.

MATERIALS AND METHODS

The experiment was carried out on Cory's shearwaters breeding in the Atlantic Ocean on Selvagem Grande (Macaronesia, Portugal: 30°09'N, 15°52'W), during June 2012. This colony, where the breeding population is estimated at 30,000 pairs and which covers the whole island (Granadeiro et al., 2006), is the largest in the world and is unusual because it is the only one where Cory's shearwaters reportedly show both nocturnal and diurnal behaviour. Treatments and release procedures were carried out using the methods already described for other experiments on procellariiforms (Benvenuti et al., 1993; Bonadonna and Bretagnolle, 2002; Bonadonna et al., 2001). All aspects of the study were performed according to guidelines established by the Centre National de la Recherche Scientifique for the ethical treatment of animals, were approved by the ethical committee during the evaluation of the Marie Curie Intra-European Fellowship proposal (SOMA) and complied with current French and Portuguese regulations. This study was authorized by the Serviço do Parque Natural da Madeira, licence number 5/2011.

At 20 nests we captured the individual incubating the egg. After measuring and ringing it, we marked it with a coloured sign on the chest using an animal marking stick for short-term marking (Raidex,

Germany). Marks were harmless and washed away after a foraging trip. Nests were then monitored daily and the marks provided an easy way to check for the shift among partners in egg incubation without continuous recaptures of the bird, as the nests in this colony are shallow burrows or open crevices in which the birds are easily visible from the exterior. On the second day after a partner's shift, we captured, measured, ringed and treated the incubating shearwaters. Birds were assigned to either the anosmic (three males and three females) or control group (four males and two females). This allowed us to select for the experiment only birds highly motivated to return to the nest after displacement because they were early on in their incubation stint. Ethical considerations constrained us to use a small sample size, but this same number of anosmic and control birds have proved sufficient to reveal significant differences in a previous study (e.g. Bonadonna et al., 2001). Birds were sexed using beak measurements according to the specific formula for the Cory's shearwater from this colony (Granadeiro, 1993).

Anosmia was induced in experimental birds by washing the olfactory mucosa with a solution of zinc sulphate eptahydrate ($ZnSO_4 \cdot 7H_2O$) at 5% (Benvenuti et al., 1993; Bonadonna et al., 2001; A. Gagliardo, J. Bried, P. Lambardi, P. Luschi, M. Wikelski and F.B., submitted). This treatment produces a reversible but complete smell deprivation by impairing the olfactory mucosa (Cancelon, 1982), while the birds can continue to breathe normally. Birds recover functional smell within some days of the manipulation, thanks to the natural regeneration of the olfactory epithelium. The zinc sulphate solution was prepared by dilution of 5 g of $ZnSO_4 \cdot 7H_2O$ in 100 ml of water. Birds were injected with 5 ml of solution into each nostril using a curved needle with a silicon rubber smoothed point, in order to reach the entrance of each olfactory chamber. This method is the most efficient way of irrigating the olfactory mucosae (Benvenuti et al., 1993; Bonadonna et al., 2001; A. Gagliardo, J. Bried, P. Lambardi, P. Luschi, M. Wikelski and F.B., submitted). Birds were held by keeping the bill open and towards the ground to allow the solution to flow out from the choanae. Control birds underwent the same treatment but the zinc sulphate solution was replaced by a physiological saline solution. Treatments were carried out in the morning and, after treatment, birds were returned to their nest to let them recover from the stress of manipulation, where they normally resumed incubation.

Releases were performed during the second night after treatment, approximately 36 h later. In this way, subjects with a low motivation to return home were excluded because the birds had sufficient time to abandon their nest in response to stress from the manipulation. At night, approximately 30 min after complete dark, treated birds were taken from the nest and transported in a cotton bag to the same release site in the colony, 100–250 m distant from the nest burrows. The island consists of a central plateau, completely surrounded by cliffs. The release site was chosen on top of the cliffs, in order to facilitate birds' take off, in a place where the home burrows were not visible either at night or during the day. The conformation of the release site obliged birds to fly to return to the nest, as it was not possible for them to home by walking. Birds were released by gently leaning the cotton bag on the ground and opening it so that the bird could exit spontaneously by walking out the bag. Birds were then left quietly alone on the ground and free to take off. This procedure was possible thanks to the absence of predators on the island. Not all the birds were released on the same night because not all the partners' shifts occurred at the same time, but for each release an equal number of control and anosmic birds was used. Because of the absence of predators and because procellariiform eggs can be left cold for days without compromise (Boersma and

Wheelwright, 1979; Chaurand and Weimerskirch, 1994), eggs were left alone in the burrows.

Burrows were checked from the next morning onward several times per day, starting from immediately before dawn, until immediately after dusk, until the released bird was back. Checking nests close to dawn and dusk allowed us to distinguish whether birds homed during the night or during daylight and at which time of the day. To avoid the additional stress of a recapture, and the possibility that homed birds might abandon the nest afterwards, birds were not weighed at their return to the nest. Ring numbers were verified by gently pulling the bird leg, without removing the bird from either the nest or the egg.

To compare homing success, we performed a Wilcoxon–Mann–Whitney test for independent samples, taking into account the number of days elapsed between the release and homing dates, as in previous studies (e.g. Bonadonna and Bretagnolle, 2002). In addition, we compared homing performance by day or by night in the two groups with a Fisher exact test. Finally, we employed a *G*-test to compare the homing success at dawn just after release (immediate homing success), applying the Yates correction because of the small sample size (McDonald, 2009). Statistical analyses were performed with the aid of R (R Development Core Team, 2011).

RESULTS

All treated birds were released during the second night after treatment, and none abandoned the nest before release. At the moment of capture, each individual was incubating its egg and apparently exhibiting normal behaviour indistinguishable from that of unmanipulated birds. The mean mass of the anosmic birds was 938.3 ± 47.6 g ($N=6$) and that of the controls was 923.3 ± 27.3 g ($N=6$; means \pm s.e.m.). A *t*-test for independent samples showed no difference in mass between the two groups ($P>0.75$).

As in previous studies, there was no difference between anosmic and control birds in the number of days after release taken to home (Wilcoxon–Mann–Whitney test for independent samples, $P=0.4$). However, a difference emerges when considering the nycthemeral distribution of homing. All control birds homed during the same night of release before dawn, but none of the anosmic birds did so (*G*-test, $P=0.027$). In contrast, five out of six anosmic birds homed

during the first day after release, two in the morning and three in the afternoon. The last anosmic bird returned to the nest in the afternoon of the third day after release (Table 1). The difference in the homing performance by day or by night was significant: all controls birds homed by night while all anosmic birds homed by day (Fisher's exact test, $P=0.002$).

DISCUSSION

Our results suggest that diurnal petrel species may need an intact and functional sense of smell to find their burrow when homing at night. Cory's shearwater is a nocturnal species that homes by night as well as by day to the colony on Selvagem Grande, where we performed the experiment. Anosmic Cory's shearwaters were unable to home in the dark and waited for daylight to return to their burrow; none of the anosmic birds, in fact, was back at the nest before the rising of daylight. By contrast, all control birds returned to their burrows during the same night of release, before dawn. Our results imply that also diurnal petrels use olfaction for homing when returning at night and that the use of olfactory cues to find the nest burrow is not a strategy exclusive to nocturnal species.

In shearwaters that may home both by day and by night, smell deprivation induced a constraint in the nycthemeral distribution of homing behaviour. When considering the homing performance in terms of number of days before homing, experimental and control groups showed no difference, in accordance with previous experiments (Bonadonna and Bretagnolle, 2002). However, when taking into account the time at which released birds were back at their nests, the different behaviour between anosmic and control birds becomes evident. Previous experiments only checked the difference in number of days elapsed before homing, without considering the possibility that different strategies could be used to home by night and by day (Bonadonna and Bretagnolle, 2002). Here, we showed for the first time that to succeed in homing, anosmic Cory's shearwaters became constrained to home in daylight, probably shifting to visual cues and employing landscape features for homing. It seems plausible that a similar shift may also have occurred in wedge-tailed shearwaters that homed despite sectioning of their olfactory nerve (Shallenberger, 1975), and further studies on this and other species would be worthwhile.

Table 1. Homing performance of birds subjected to zinc-sulphate-induced anosmia and control birds, specifying whether they homed before dawn, in the morning or in the afternoon till dusk

Bird	Release date	No. of days after release						
		0			1/2	3		
		Dawn	Morning	Afternoon		Dawn	Morning	Afternoon
Anosmic birds								
1	20/6	–	–	+				
2	20/6	–	–	+				
3	21/6	–	+					
4	21/6	–	+					
5	22/6	–	+					
6	23/6	–	–	–	–	–	–	+
Control birds								
A	20/6	+						
B	20/6	+						
C	21/6	+						
D	21/6	+						
E	22/6	+						
F	23/6	+						

+, present at burrow; –, absent from burrow. Days 1 and 2 are combined for simplicity.

In our experiment, all treated birds, both control and anosmic, were apparently highly motivated to home as all but one experimental bird returned to its burrow within the first day of release. Only one bird homed later, after 3 days, but this is common in this kind of experiment, even for control birds (Benvenuti et al., 1992; Bonadonna and Bretagnolle, 2002; Bonadonna et al., 2001), and may be due to an individual response to stress. Anyway, the late-returning shearwater was still impaired in its smelling capabilities at the moment of homing, because the olfactory epithelium needs longer to re-establish normal functioning at the concentration of zinc sulphate used (Cancalon, 1982), and this bird also homed during the day. We also note that the diurnal activity of shearwaters at the colony is generally limited to the afternoon, when birds start to arrive from foraging trips and the colony starts to become crowded. In the morning, by contrast, flying birds are observed only very rarely. To this extent anosmic birds that homed in the afternoon arrived during the 'normal' diurnal homing time, while those that homed during the morning displayed a particularly strong motivation to return to the burrow.

Our results also show that zinc-sulphate-induced anosmia is an effective method of inducing complete anosmia without influencing normal behaviours, such as the motivation to home or to incubate. This finding is important because experiments inducing anosmia have been previously criticized as unreliable because smell deprivation might modify normal behaviour and, in particular, impair the motivation to home (Wiltschko, 1996), a criticism that has potential implications for interpreting the delaying effects of anosmia in terms of the mechanisms of homing. Zinc sulphate treatment is a standard protocol for olfactory deprivation both in homing pigeons and in petrels (Benvenuti et al., 1992; Benvenuti et al., 1993; Bonadonna and Bretagnolle, 2002; Bonadonna et al., 2001; Guilford et al., 1998). In fact, this treatment has an advantage over other methods because it induces complete anosmia but only temporarily, unlike nerve sectioning, and it allows birds to continue to breathe normally, unlike nostril plugs. In addition, our data now clearly show that the zinc sulphate treatment does not change the motivation to home, so results obtained with this method are fully reliable.

In conclusion, our results show that anosmic shearwaters were unable to home by night and were constrained to home during daylight. These findings strongly suggest that olfaction provides the essential cues for nocturnal homing and that even diurnal petrels homing by night use olfaction to find their burrows. Our results also suggest that petrels may use different orienting cues and shift from one to another according to conditions. The next step will be to track precisely the homing paths in order to understand whether anosmic birds rest at sea until morning or whether they try to home during the night, but without succeeding, and then home during the day. Tracking anosmic birds' homing might also allow an understanding of the stage at which odours are critical, whether in short-distance orientation or in own-nest recognition, as well as provide evidence concerning the use of visual landmarks for orientation.

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AUTHOR CONTRIBUTIONS

G.D.A. and F.B. designed the experiment, collected and analysed the data and revised the article. G.D.A. drafted the manuscript.

COMPETING INTERESTS

No competing interests declared.

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