Evidence for olfactory learning in procellariiform seabird chicks

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Burrow nesting procellariiform seabirds use olfactory cues for both foraging and nest recognition. As chicks, burrow nesters develop in the dark, but are exposed to both prey-related and individual-specific scents through contact with their parents. This exposure suggests that chicks may have the opportunity to learn odours while still in the nest. In this study, we examined whether exposure to odourants during development might influence olfactory search behaviour expressed later in life. To test this idea, we exposed eggs of thin-billed prions *Pachyptila belcheri* to a rosy-scented novel odour (phenyl ethyl alcohol, PEA) or a control (water) just before hatching; chicks were then tested with these odours in a simple wind tunnel. Prior to fledging, subjects who had received pre-exposure to PEA displayed head sweeps nearly twice as frequently as control birds did when presented with PEA. This study demonstrates that under natural rearing conditions, procellariiforms learn odour characteristics of their rearing environment in the nest.

Petrels and albatrosses nest on remote islands either on the ground or in burrows, depending on the species. All procellariiforms are highly pelagic, often foraging in locations hundreds of kilometres from breeding colonies. These birds are consequently tied to land only to breed and rear their young. Breeding pairs lay a single egg, which the parents incubate in shifts for ∼45 days. Upon hatching, chicks live underground in complete darkness for another 45 days until they fledge. Chicks are nourished on regurgitated stomach oils for much of this time and have no exposure to live prey. Approximately seven to ten days before fledging, the parents abandon their offspring to survive independently. Whereas other types of seabirds spend months tutoring or provisioning their fledglings (e.g. Simmons 1967, Varoujean et al. 1979), procellariiform fledglings are left to find prey in a vast ocean on their own without parental instruction (Warham 1996).

We and others have previously shown that adult burrow-nesting petrels are highly responsive to odour cues at sea (reviewed by Nevitt 2008). For example, results from at-sea experiments conducted in the sub-Antarctic show that burrow nesting species such as prions Pachyptila spp. and storm petrels (e.g. Wilson's *Oceanites oceanicus*) are attracted to scents associated with zooplankton grazing (e.g. dimethyl sulfide, DMS), and we have speculated that burrow nesting species, in particular, make use of these scented compounds to opportunistically forage on ephemeral prey patches (Nevitt et al. 2004). For such species, finding prey efficiently is particularly critical, since they tend to use more maneuverable but less efficient flight styles than other, ground-nesting species (see discussion in Van

Buskirk and Nevitt 2008). In previous work, we have found that procellariiform chicks respond to DMS and other prey-related odours (Cunningham et al. 2003, 2006, Bonadonna et al. 2006). Adults consume prey items that contain precursors to DMS (dimethylsulfonioproponate, DMSP; reviewed in Nevitt 2008), and these precursors could transfer to the embryo to influence the development of olfactory preferences. In chickens *Gallus domesticus* fishy smelling and tasting eggs have been reported after hens have been fed Omega-3 polyunsaturated fatty acids, suggesting that scented compounds might be passed to the egg prior to laying (Leskanich and Noble 1997). We have also noted that adult procellariiforms sometimes smell of phytoplankton when returning from sea, suggesting that their plumage may carry prey-related scents that could be transferred to the egg during incubation. Together, these observations led us to hypothesize that parents may be able to shape olfactory-based behaviours indirectly by exposing offspring to odours during embryonic development (e.g. Burne and Rogers 1999). Based on this reasoning, we tested whether pre-exposure to a novel odourant in the egg influenced the fledgling's search response to that odour later on.

Materials and methods

We tested this hypothesis at Mayes Island (49° 28′S, 69° 57′E) in the Gulf of Morbihan, Kerguelen archipelago, using a burrow-nesting procellariiform, the thin-billed prion *Pachyptila belcheri* (Matthews; Fig. 1). Seven to ten days prior to hatching, we applied 1 ml of either scented

Figure 1. A 22-day-old thin-billed prion chick. Photo © by Petra Quillfeldt, 2007.

(phenyl ethyl alcohol, PEA: Sigma-Aldrich, St. Louis, Missouri, USA, a rosy-smelling novel odourant, 0.1 μM), or control (distilled water) solutions to 40 eggs (20 per treatment) using an artist's paint brush (Sneddon et al. 1998). The eggs were painted every other day for a total of 4-5 applications per egg, depending on hatch date. We have previously shown that petrel chicks can detect this odourant (Cunningham et al. 2003), but are relatively unresponsive to it in behavioural trials (Cunningham et al. 2006). Of these eggs, 12 PEA-treated and 11 control-treated chicks hatched; hatch failure fell within normal limits (Weimerskirch pers. com).

Approximately 40 days (range: 37-42 days, mean $+/-$ SE: 39 $+/-$ 0.6 days) post hatching, we tested the response of chicks to odours in a flow-through arena (0.8 m × 0.6 m; 0.3 m high) constructed of plexiglass. The arena was set up inside a well-ventilated field hut (1.5 m × 1.5 m × 2.5 m) located ~ 500 m from the colony, and has been described elsewhere (Cunningham et al. 2006). Briefly, airflow (0.6 – 0.7 m/s) was generated by two tubeaxial fans mounted on each side of a pressure box (air volume: 3 m^3/min). Fans drew in ambient air from outside of the hut through 9 cm dia. ducting. To reduce turbulence in the testing arena, the incurrent air was filtered through egg-crate plastic lined with fine nylon organdy cloth.

Trials were conducted between 10:00 and 15:00 (17.2 $\pm$ 0.5° C). Due to logistic constraints, chicks had to be tested before they were fully ambulatory. Thus, our goal was not to present the odour as a discrete plume that could be followed to its source, but rather as a presence/absence of odour in the arena. To achieve this test condition, a glass dish (radius, 71mm; height, 41 mm), was placed upwind from the chick. The glass dish contained a cotton swab (1 cm × 5 cm; 0.5 cm thick), which was saturated with 1 ml of the stimulus solution (either 1 μM PEA or a distilled water control). Odour or control presentations were presented sequentially and the presentation order was balanced between trials.

Behavioural trials were performed in the dark and videotaped under infrared using a Sony camcorder (model DCR-TRV30). Chicks were tested one at a time. To begin a trial, a chick was positioned inside the arena at a predefined 'start' point near the center of the arena.

Following a 1 min acclimation period, we removed the lid of the dish, exposing the swab to the airflow. The chick was then videotaped for at least 3 min. Following the trial, the stimulus dish was replaced and the chick was moved back to the 'start' point. Response to the second stimulus was video recorded using the same procedure. The chick was then weighed, measured (for wing length, tarsus length, and bill length), and returned to its burrow. Each chick was used only once. Chicks (n = 3) that appeared stressed or walked out of the arena were removed from the experiment.

For analysis, video images were projected onto a Sony Trinitron colour video monitor (model PVM-1351Q). Data were scored by a naïve, 'blind' observer using Jwatcher software (Blumstein, Evans and Daniel, Animal Behaviour Laboratory, Macquarie University, Australia). Behaviours initially scored included head sweeps, biting, preening and walking events. Preening, walking, and biting events were noted in <40% of the birds tested, and so statistical analysis was limited to head sweeps using one-tailed paired Student's t-tests. One-tailed tests were used because we already established that petrel chicks are more active to food odors, (Cunningham et al. 2006), thus, we predicted that search behavior would increase to PEA.

Results

Although most chicks were still highly immobile and covered in down (tarsus length: 32.7 $+/-$ 0.4 mm; wing chord: 137.9 $+/-$ 5.5 mm, weight: 143 $+/-$ 8.0 g), nearly every chick responded to test stimuli by wagging its head from side to side in broad, sweeping motions around the body (PEA pre-exposed birds: 10/10; control pre-exposed birds: 9/10). In the presence of PEA, PEA-pre-exposed birds performed head sweeps significantly more frequently in response to PEA than to control distilled water (Fig. 2A, Paired Student's one-tailed t-test, t = 1.88; p < 0.05). By contrast, control pre-exposed birds swept their heads at the same rate under these two test conditions (Fig. 2B, t = − 0.08, p = 0.4702).

Discussion

The results presented here suggest that pre-exposure to PEA influences the degree to which chicks sweep their head in response to this odourant. Head sweeping behaviour is associated with olfactory search in other petrels in both natural and experimental settings (Grubb 1974) and is exhibited in a variety of animals that are adapted to sample odours on or close to the substrate, including mammals, and reptiles (see Li et al. 2001). Head sweeping precedes body turning in adult petrels as they search for their nest sites on land (Nevitt pers. obs.), while at sea, turning is likely to focus activity towards the odour source during an area-restricted search (Nevitt 2008). To our knowledge, head sweeping is exhibited by adult petrels only when they walk on land, but is also reminiscent of the characteristic zigzag search behaviour petrels use to forage at sea (see discussion in DeBose and Nevitt 2008).

Both pre- and post-natal chemosensory learning have been described in a number of different species, including
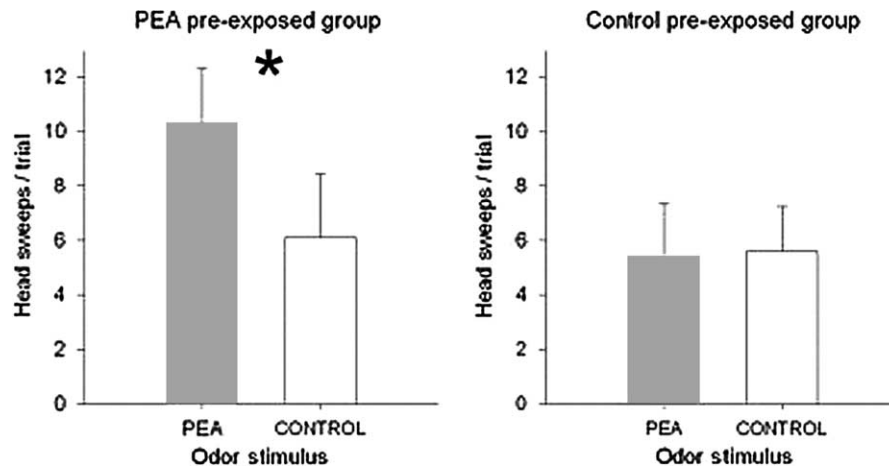
Figure 2. Mean (± SEM) number of head turns by thin-billed prion chicks pre-exposed to (A) PEA or control (B) distilled water. Black bars indicate responses to PEA stimulus presentations whereas white bars indicate responses to control stimulus presentations. The asterisk indicates a significant difference ($p < 0.05$, see text).

humans (reviewed by Hudson 1999). In some cases, olfactory pre-tuning has been shown to provide offspring with an advantage in finding food (reviewed by Miller and Spear 2008), and this may be particularly important for petrels that must leave the nest prepared to forage in a vast ocean. Rabbit pups *Oryctolagus cuniculus*, for example, imprint to the scent of food-related odours expressed in the milk of their mother (Altbacker et al. 1995; Semke et al. 1995), while ferrets *Mustela nigripes* develop a preference in adulthood for the odour of food items received as kits (Vargas and Anderson 1996).

Although no studies have directly investigated perinatal odour learning in wild birds, Sneddon et al. (1998) demonstrated that, in a laboratory setting, painting chicken eggs with strawberry odours reduced chicks' natural aversion for strawberry scented water. In our study, we cannot determine when learning occurred since chicks were brooded by their parents under natural conditions, and could have been exposed to scents from eggshells before, during or after hatching. During this time, a chick would normally be exposed to odours associated with feeding as well as to scents permeating the parents' feathers, suggesting that the brooding period may be a likely time for olfactory information to be transferred to the chick (Sneddon et al. 1998).

In conclusion, while the olfactory abilities of procellariiforms have been recognized for some time, this study is the first to directly explore the underlying mechanisms for how these abilities are shaped by early experience. Building on studies with domestic chickens (Mabayo et al. 1996; Burne and Rogers 1999; Porter et al. 1999; Jones et al. 2002), our results demonstrate that thin-billed prion chicks are capable of learning odour cues while still in the egg. These results add further support to the suggestion that procellariiform fledglings have the ability to learn olfactory characteristics of the outside world even before they leave their burrow for the first time.

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