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Olfactory conditioning experiments in a food-searching passerine bird in semi-natural conditions

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Abstract

Because passerine birds have a very small relative olfactory bulb size, they have been considered to have weak olfactory capacities for decades. Recent investigations however suggest that breeding female blue tits (*Parus caeruleus*) are sensitive to lavender odour in the reproductive context of building and maintaining the nest. Here, we present results of an olfactory conditioning experiment in blue tits held in semi-natural conditions during the breeding season. We show that captive male blue tits, trained to associate lavender odour with a food reward, are more attracted to an empty feeder box emitting lavender odour than an odourless empty feeder box. Females did not distinguish significantly between empty feeders with and without lavender odour during the test phase, although they responded positively at the end of the training phase. These results suggest that male blue tits can use olfaction in a context not related to nest building. Additional experiments will be required to better understand the observed sex differences in response to the experimental set up, and in what context free-ranging individuals use olfaction.

Keywords: Blue tit; Conditioning; Olfaction; *Parus caeruleus*; Passerine

1. Introduction

The importance of bird olfaction has been debated for decades. Since the 1960s, however, an increasing number of studies indicates that the olfactory apparatus of various avian species is similar to that of other vertebrates (reviewed by Roper, 1999). The use of olfaction has now been demonstrated in biologically relevant contexts, including orientation in homing pigeons (reviewed by Wallraff, 2004), food finding in kiwis (Wenzel, 1968), turkey vultures (Houston, 1986), and petrels (Hutchison and Wenzel, 1980; Nevitt et al., 1995; Nevitt, 2000; Cunningham et al., 2003), and recognition of familiar odours in domestic chicks (Burne and Rogers, 1996; Marples and Roper, 1996; Porter et al., 1999) and petrels (Bonadonna et al., 2003a,b, 2004; Bonadonna and Nevitt, 2004).

Edinger (1908) and many others (reviewed by Roper, 1999) assumed that the relative olfactory bulb size (ROBS), i.e. the ratio olfactory bulb/brain hemisphere of each species is related to the importance of its olfactory capabilities. According to a comparative

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study of the ROBS of 108 species from 21 avian orders, Passeriformes, together with Psittaciformes, possess the smallest ROBS of all studied orders (Bang and Cobb, 1968). Thus, in theory, passerine birds and parrots should have weak olfactory capacities. However, Clark and colleagues demonstrated with different experimental approaches that a passerine bird, the European starling (*Sturnus vulgaris*), is sensitive to volatile compounds emitted by the plants used for nest building (Clark and Mason, 1987; Clark and Smeraski, 1990). In addition, recent conditioning experiments indicate that some parrots are able to associate an odour with a food reward (Roper, 2003; Hagelin, 2004). In particular, Roper (2003) successfully trained two captive yellow-backed chattering Lories *Lorius garrulus flavopalliatus* to distinguish an odorant dispenser containing nectar from an odourless dispenser containing water. This study suggests that even some birds with small olfactory apparatus are able to use olfaction to find a food resource, and provides a simple experimental design to test for birds' capabilities to associate odours and resources.

In the blue tit from Corsica *Parus caeruleus ogliastrae*, a small hole-breeding passerine bird, females deposit fragments of several aromatic herbs (e.g. lavender, mint) on the top of the nest cup between the onset of egg laying and the time chicks leave the nest (Lambrechts and Dos Santos, 2000). During the incubation and rearing period, they regularly bring new aromatic herb fragments in the nest, also after experimental removal (Lambrechts and Dos Santos, 2000). Field experiments indicate that free-ranging female blue tits use olfactory cues in the nest cavity to determine the frequency with which they replenish the nest cup with fresh aromatic herb fragments (Petit et al., 2002). In a preliminary conditioning experiment, captive breeding Corsican blue tits have been successfully trained to associate lavender odour with a food reward, although the small sample size did not allow for further analysis, especially for comparison between male and female behaviours (Fossette et al., unpublished).

Here we present the results of an additional conditioning experiment, where captive Corsican and continental blue tits during the breeding period were trained to associate and use odour cues to find a food reward. This is, to our knowledge, the first study that employed olfactory conditioning in a non-domesticated passerine species in semi-naturalistic settings.

2. Materials and methods

2.1. Birds

Thirty-nine blue tits (20 males and two females from Corsica, 17 females from the surroundings of Montpellier, France) were used for the conditioning experiments carried out in outdoor aviaries between 19th June and 3rd July 2004. Birds were held in large outdoor aviaries (27 m^3) at the field station of the Centre d'Ecologie Fonctionnelle et Evolutive Laboratory (CNRS), Montpellier, France (see Lambrechts et al., 1997, 1999 for description of the aviaries). All birds had been held in captivity for more than 3 months. Tits were fed with a diet of cake, apple, mealworms (larvae of *Tenebrio molitor*) and sunflower seeds from a permanent feeder. Food and fresh water were delivered ad libitum. The survival probabilities and physical condition of the captive blue tits were at least as high as those observed in the free-living populations (cf. Lambrechts et al., 1997, 1999; Braillet et al., 2002).

2.2. Conditioning experiment and training

The conditioning apparatus used to train the birds to associate food with an odour cue was a tripod of 1.30 m high supporting a horizontal perch of 60 cm long. The horizontal perch was equipped with two identical cylindrical, non-transparent plastic feeder boxes (tubes of 30 cm high and 10 cm diameter with two 2.5 cm opposite holes and a removable non-transparent lid). The distance between the two boxes fixed on the horizontal perch was 60 cm. These feeder boxes were different in form and shape from the feeder that was permanently available in the aviaries. The content of the two feeder boxes was visible and accessible to tits only when they perched on one of two holes and looked inside the box. Ringworms (*Galleria sp.*), which constitute a very attractive kind of food to captive blue tits, were used as reward in our conditioning experiment.

During a training session lasting 10 days, live ringworms were associated with odour of lavender. Per tripod, one feeder box, called "odour+", contained both five ringworms and lavender odour diffused by two drops on a piece of pottery (4 cm^2) of both pure essential oils of *Lavandula angustifolia* and *L. latifolia.* The lavender oil was not in contact with ringworms. The other feeder box, called "odour−", contained a similar piece of pottery without lavender odour and without ringworms. Tits could not see the experimenter preparing the feeder boxes. The position of the feeder boxes on the tripod was randomly assigned daily. In each aviary the pair of feeder boxes used was changed daily. Feeder boxes assigned to be without odour were never contaminated with lavender odour during previous trials.

To pre-train tits to find ringworms in the feeder boxes, we presented boxes without a lid during 3 days (1 h per day in each aviary), so that subjects could see the feeder content (days −2 to 0). During the pre-training phase we did not quantify bird behaviour towards the feeder boxes. Qualitative observation revealed that birds mostly entered the boxes from the top and not from one of the two holes.

Once pre-trained to visit feeder boxes without a lid, birds were trained to visit the boxes with a lid during the next 7 days. During the training phase (days 1–7), the feeder boxes were presented 15 min per day in each aviary and one observer (AM) quantified bird behaviour during feeder visits. These observations prior to the test phase allowed us to check whether the birds: (1) quickly learned to enter the feeder boxes through one of the two holes, which was the case, and (2) were more attracted to the feeder box containing both the odour and the food reward than the odourless feeder box not containing a food reward. Birds that were efficiently trained should be significantly more attracted to the feeder box containing both the odour cue and the food reward at the end of the training phase.

We used two kinds of behaviour to determine which of the two feeder boxes was chosen and visited most during the observation periods. Every time a given bird: (1) put its head in the hole of a box, and/or (2) entered a box, the choice between the feeder boxes was considered done. The birds sometimes perched on the lids of the boxes before making the choice to inspect them or not, but most of the time they chose them while flying around or perching on surrounding vegetation.

2.3. Test phase

Blue tits were tested during eight consecutive days following the training phase (days 8–15). During each day of the test phase, two feeder boxes with a lid, but without ringworms, were prepared. Tits could not see the experimenter preparing the boxes. One of the two feeder boxes, called “odour+”, contained lavender odour. The other box, called “odour−”, did not contain lavender odour.

In a preliminary experiment (Fossette et al., unpublished) the feeder boxes were presented to the birds during the test period in the same way as during the training period (i.e. with the feeder boxes on the tripod). Here, the two feeder boxes were placed about 3 m apart on the ground. Our goal was to determine whether tits would still be more attracted to the “odour+” box when forced both to feed on the ground and to detect lavender odour at a distance from its source. Preliminary observations with captive blue tits showed that they were also attracted by feeder boxes placed on the ground (S. Fossette, pers. commun.).

In addition, in the preliminary experiment carried out by Fossette et al. (unpublished), reinforcement was performed 4 days after the beginning of the test, when tits were still significantly attracted to the “odour+” feeder box. Consequently, the duration of the tits’ response to olfactory conditioning could not be established from that experiment. In our experiment, no reinforcement was performed during the test phase, so that we were able to test attraction to the “odour+” box during eight consecutive test days.

The same observer (AM) quantified bird behaviour for 15 min after the feeder boxes were placed on the ground in each given aviary. We expected that tits, having associated lavender odour with presence of ringworms, would approach feeder box “odour+” first, and would visit box “odour+” more often than box “odour−”. To stimulate tits visiting feeder boxes, mealworms were removed from the permanent feeder during each 15 min test period. Birds still had the option to eat cake, apple, or sunflower seeds that were available in the permanent feeder during the test phase, so that they were not penalised by the total absence of food. Most of the time birds first perched somewhere on the ground and then approached one of the feeder boxes before choosing to inspect it or not. Only a few times did the birds directly perch on the lid of a box before making a choice.

2.4. Statistical analysis

Some individuals did not visit feeder boxes during some observation periods. Consequently, to obtain sufficient sample sizes for analyses, we pooled the visits

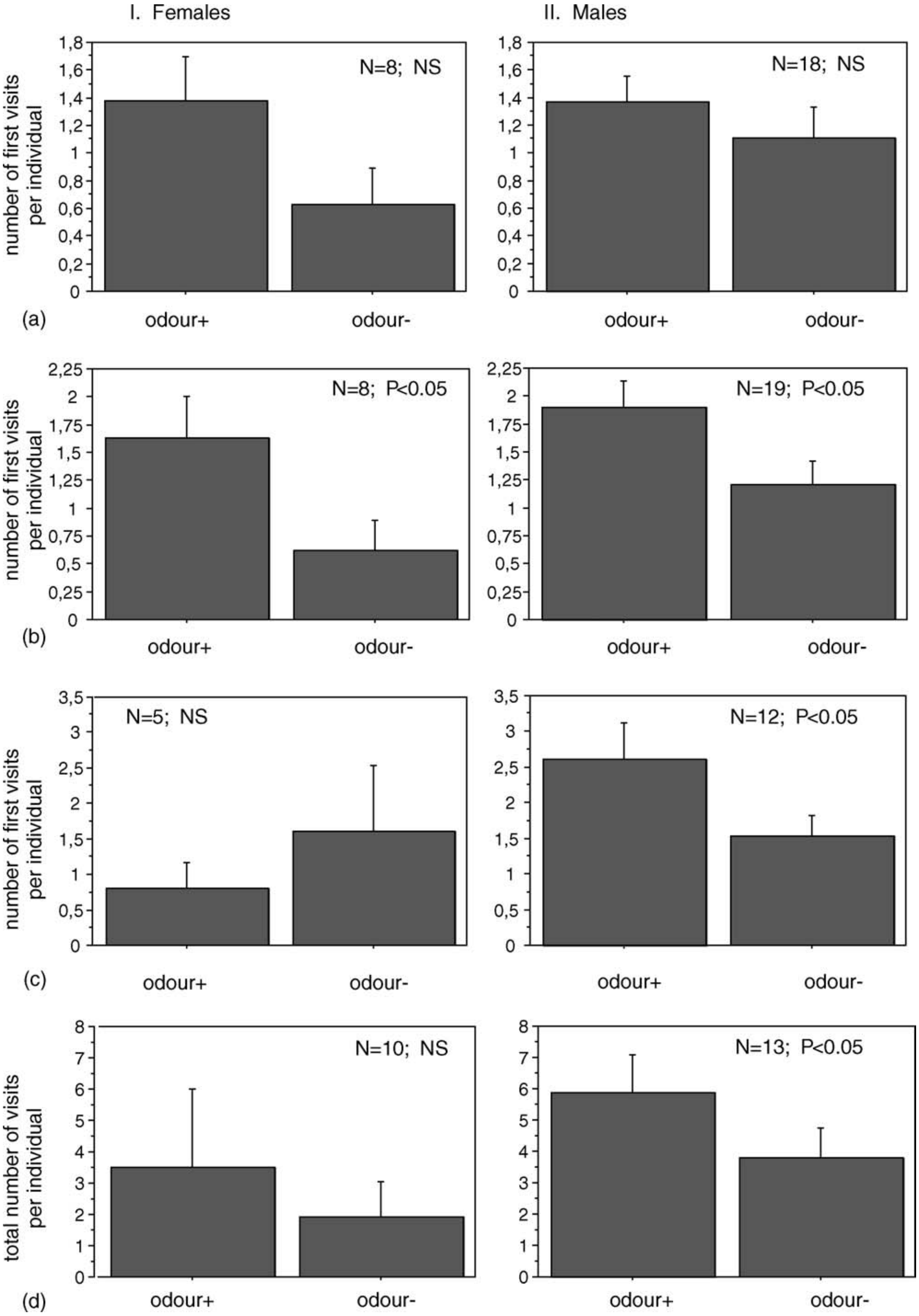

Fig. 1. Number of first visits per individual (mean ± S.E.) during: (a) the first three training days, (b) the last four training days, (c) the test phase, and (d) total number of visits per individual (mean ± S.E.) during the test phase to the feeder boxes with lavender odour (odour+) and without lavender odour (odour−). I, females; II, males; *N*, number of individuals (i.e. number of paired values to which the Wilcoxon's signed rank test was applied). Results of one-tailed Wilcoxon's signed-rank tests are indicated (NS, non significant; *P*, *P*-value).

to feeder boxes for a given individual across: (1) the first three training days, (2) the last four training days and (3) all test days.

For each period, the numbers of visits to feeder boxes with and without lavender odour were compared using a one-tailed Wilcoxon's signed rank test. This test was applied to the number of visits to the "odour+" and "odour−" feeder boxes (paired data) made by individuals that visited at least one of the feeder boxes once. One-tailed tests were performed for three reasons. First, a preliminary experiment suggested that captive blue tits, when previously conditioned, were more attracted to an empty feeder box containing lavender odour than an odourless feeder box (Fossette et al., unpublished). Second, the observations carried out during the training phase indicated that tits did not particularly avoid the feeder box containing lavender odour. Third, the aviary experiment was carried out during the reproductive period, i.e. when the tits bring lavender fragments to the nest in Corsica (see Section 1).

For each period, the numbers of times that birds visited first the "odour+" and the "odour−" feeder boxes were compared. Aviaries contained both a male and a female blue tit; an individual could thus potentially copy food-searching behaviour of its mate finding a food reward. Therefore, in order to avoid "leader-follower" situations, we took into account the first visit of a bird only when it was the first individual of its aviary to explore the feeder boxes. For the test period, the total numbers of visits made to the "odour+" and "odour−" feeder boxes were also compared. Food rewards were however not provided during the test, so copying behaviour was not rewarding. Regarding the total number of visits to each feeder box, we therefore considered all visits made by a given individual across the whole test period. The individual preference of each bird for one of the two feeder boxes was tested by a binomial test, applied to the total number of visits made by each given bird to the "odour+" and the "odour−" feeder boxes during the test phase.

3. Results

During the training phase, 20 males and 17 females visited the feeder boxes. The first individual to visit the feeder boxes was more often a male than a female (Mann–Whitney U-test, $z = -3.931$, $P < 10^{-4}$). Males also made a significantly greater number of visits to the feeder boxes than females (Mann–Whitney U-test, $z = -1.968$, $P = 0.049$). During the first three training days, neither the males nor the females visited first more often the feeder box with food and lavender odour than the odourless empty feeder box (Wilcoxon's signed rank test, one-tailed, NS) (Fig. 1a). During the last four training days, however, birds of both sexes visited first significantly more often the feeder box that contained food and lavender odour than the odourless empty feeder box (Wilcoxon's signed rank test, one-tailed, $P < 0.05$ for both sexes) (Fig. 1b), which indicates that training was successful.

During the test period, 13 males and 10 females visited the feeder boxes. The number of individuals visiting the boxes constantly decreased over the succession of days. The first individual to visit the feeder boxes was more often a male than a female (Mann–Whitney U-test, $z = -2.946$, $P = 0.003$). Males also made a significantly greater number of visits to the boxes than females (Mann–Whitney U-test, $z = -2.512$, $P = 0.011$). One male visited first significantly more often the "odour+" than the "odour−" feeder box ("odour+" = 6, "odour−" = 0, binomial test, $P < 0.05$). As a whole, male blue tits visited first significantly more often the "odour+" than the "odour−" feeder boxes (Wilcoxon's signed rank test, one-tailed, $P < 0.05$). The difference was not significant for females (Wilcoxon's signed rank test, one-tailed, NS) (Fig. 1c). One male and one female made a significantly greater total number of visits to the "odour+" feeder box than to the "odour−" feeder box during the test phase (male: "odour+" = 13, "odour−" = 1, binomial test, $P < 0.001$; female: "odour+" = 26, "odour−" = 12, binomial test, $P < 0.05$). As a whole, males made a greater total number of visits to the "odour+" than the "odour−" feeder box (Wilcoxon's signed rank test, one-tailed, $P < 0.05$), whereas females did not (Wilcoxon's signed rank test, one-tailed, NS) (Fig. 1d).

4. Discussion

Here we provide experimental evidence for the existence of olfactory abilities in males of a non-domesticated passerine bird, using an operant conditioning experiment in semi-naturalistic outdoor aviaries during the breeding season.

European starlings are known to be sensitive to plant odour cues at the time these cues are used to find resources, that is during the reproductive period when parents, especially males, look for herbs to build nests (Clark and Mason, 1987). Olfactory abilities to detect these plant odours seem to decrease outside the breeding season in this passerine bird (Clark and Smeraski, 1990). Our experiment was carried out during the breeding season, i.e. a period that free-ranging female blue tits incorporate lavender and other aromatic plants in the nest, and use volatile compounds of these herbs to maintain an aromatic environment in the nest cavity (Petit et al., 2002). Here, we show that male blue tits from Corsica are sensitive to lavender odour as well. Because males do not contribute to nest building in free-ranging blue tit populations, this finding suggests that they can use olfaction in a context other than nest building. For instance, male olfaction might be used to detect the volatile compounds emitted by caterpillar-damaged leaves to find more rapidly the key prey essential to raise the chicks. Mäntilä et al. (2004) recently showed experimentally that males and females of a small passerine bird, the willow warbler *Phylloscopus trochilus,* might be sensitive to volatile compounds produced by insect-damaged mountain birches, and did not exclude that, besides vision, olfaction could be involved.

The success of laboratory experiments with non-domesticated animals depends on the abilities of subjects to accept new artificial objects and captivity (e.g. Lambrechts et al., 1999). During the aviary experiments, males generally behaved differently from females. They visited the feeder boxes significantly more often than females during the training phase, and thus had a better opportunity to associate the presence of ringworms with lavender odour. Such a sex-difference in behaviour may be due to either males dominating females (e.g. Piper, 1997) and/or the fact that females are more careful or reticent in approaching new objects. The observation that the only female that visited the feeders frequently during the test phase was significantly more attracted to the feeder with lavender odour seems to support the idea that females were less well conditioned than males, although they responded positively at the end of the training phase. Another interpretation could be that during the breeding season, females already use olfaction and aromatic cues in the context of nest building. It might therefore be more difficult for them to apply olfaction to the feeding context. Whatever the cause, females in the presence of a male did not visit the feeder boxes often enough to allow for clear conclusions on their olfactory abilities in a food-searching context.

To conclude, our study supports the broad hint, first given by experiments of Petit et al. (2002), that blue tits use their sense of smell, despite their small olfactory neuroanatomy. Clearly, other experiments will be required to find out why females behaved differently from males in a conditioning context, and how, and to what extent, free-ranging passerine birds use olfaction to find natural resources essential for reproduction and survival.

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