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Mammalian Irritants as Chemical Stimuli for Birds: The Importance of Training

J. RUSSELL MASON AND LARRY CLARK
USDA/Animal and Plant Health Inspection Service, Animal Damage Control, Denver Wildlife Research Center, % Monell Chemical Senses Center, 3500 Market Street, Philadelphia, Pennsylvania 19104, USA

Although the morphological organization of the peripheral trigeminal system in birds is not very different from that found in mammals (Dubbeldam and Veenman 1978), marked functional differences appear to exist (Kare and Mason 1986, Mason et al. 1989, Norman et al. 1992). For example, birds rarely avoid substances that are irritants for mammals, even though the avian trigeminal system is responsive to chemical stimuli (Walker et al. 1979, Mason and Silver 1983). Rock Doves (*Columba livia*), Red-winged Blackbirds (*Agelaius phoeniceus*), European Starlings (*Sturnus vulgaris*), and Gray Partridges (*Perdix perdix*) are indifferent to 10,000 ppm ammonia (Soudek 1929, Mason and Otis 1990). Parrots (*Amazona* spp.; Mason and Reidinger 1983a), Rock Doves (Szolcsanyi et al. 1986), Red-winged Blackbirds (Mason and Maruniak 1983), European Starlings (Mason et al. 1991a), House Finches (*Carpodacus mexicanus;* Norman et al. 1992), and Cedar Waxwings (*Bombycilla cedrorum;* Norman et al. 1992) are indifferent to 10,000 ppm or more of capsaicin, the pungent principle in *Capsicum* peppers. Red-winged Blackbirds and European Starlings are indifferent to 10,000 ppm gingerol and zingerone, the irritants present in ginger (*Zingiber officinale*), as well as piperine, the active ingredient in black pepper (*Piper nigrum;* Mason and Otis 1990).

The indifference that birds exhibit towards mammalian irritants might reflect insensitivity. Alternatively, indifference might indicate a relatively high tolerance for these substances independent of sensation. The present experiment was designed to address this issue by investigating whether birds could be trained to avoid mammalian irritants.

Methods.—European Starlings were decoy-trapped in May 1993 near Sandusky, Ohio, and air-shipped to the Monell Chemical Senses Center. Upon arrival, the birds were individually caged (61 × 36 × 41 cm) under a 11:13 h light : dark cycle (lights on 0700-2000

EST). Prior to testing, feed (Purina Flight Bird Conditioner, Purina Mills, St. Louis, Missouri) and tap water were provided *ad libitum*.

Capsaicin (CAS# 404-86-4), piperine (CAS# 94-62-2), zingerone (CAS# 122-48-5), ethyl ether (CAS# 60-29-7), and lithium chloride (CAS# 7447-41-8) were purchased from Sigma Chemical Company (St. Louis, Missouri). Turpentine (CAS# 8006-64-2) was provided by a manufacturer (Union Camp, Princeton, New Jersey), and was included as a stimulus because it contains a variety of terpenoid compounds that are broadly repellent to mammals (Harborne 1991). Also, turpentine has been used as a seed treatment to reduce sprout-pulling by wildlife, including passerines (Mason and Bonwell 1993).

To assure an even distribution in feed, capsaicin, piperine, and zingerone were dissolved in 2 ml of ethyl ether, and then mixed with feed to produce high (1.0% mass/mass [m/m]) and low (0.1% m/m) concentrations. The adulterated feed samples were placed under a ventilation hood for 48 h to evaporate the ether (Jakubas et al. 1992). Turpentine was not diluted with ethyl ether, but was mixed directly with feed to produce a high (1.0% m/m) and a low (0.1% m/m) concentration. Control feed samples (for all stimuli but turpentine) were prepared by mixing ethyl ether with plain feed. Untreated Flight Bird Conditioner served as the control in tests with turpentine-adulterated feed. All feed samples were stored at −17°C in closed containers until 30 min prior to use.

During July 1993, we randomly assigned 120 European Starlings to four cohorts. During a four-day pretreatment period, all birds were presented with two food cups at 0800. Both cups contained 20 g of control feed (ether-treated or plain). After 2 h, the feed remaining in each cup was weighed. Between 1000 and 2000, all birds were provided with free access to untreated feed and tap water. Overnight (1700–0800), birds were food deprived. The food-deprivation regime remained in effect throughout the experiment.

At the end of the pretreatment period, mean consumption was used to assign the birds within each cohort to six counterbalanced groups (n = 5/group). The six groups were randomly paired and, within pairs, one group was assigned to the experimental condition while the other served as a control.

On the day of conditioning, all birds were given a cup containing 20 g of treated feed. Different cohorts were presented with different irritants and, within cohorts, different group pairs were presented with different stimulus concentrations (1.0% or 0.1% m/m). After 2 h, the feed remaining in the cup was weighed, and the birds in the experimental groups were gavaged with lithium chloride (2 mg/kg body mass; Mason and Reidinger 1983b, Mason and Silver 1983). Control groups were gavaged with distilled water.

A four-day test period immediately followed the day of conditioning. On each of these days, all birds were given two-cup tests between 0800 and 1000. One cup contained 20 g of the appropriate control feed, while the other contained 20 g of feed adulterated with the irritant and stimulus concentration experienced on the day of conditioning. To control for side biases, cup positions on each day for each bird was randomly determined.

For data analysis, mean pretreatment-preference ratios were calculated by dividing overall consumption from the left (or right) cup by overall consumption from both cups during the pretreatment period. Mean treatment-preference ratios were calculated by dividing overall consumption of treated feed by overall consumption from both cups during the treatment period.

Ratios for each irritant stimulus were evaluated separately in two-factor analyses of variance (ANOVA) with repeated measures over periods. The independent factor in these analyses was groups. Subsequent to the omnibus procedures, Tukey tests (Winer 1971) were used to isolate significant differences among means ($P < 0.05$).

Results.—For capsaicin, there was a significant interaction between groups and periods ($F_{5,23} = 4.3$, $P < 0.02$). Posthoc tests showed that the mean treatment-preference ratio for the experimental group given the high capsaicin concentration was significantly lower than: (a) the mean pretreatment-preference ratio for this group, and (b) the mean treatment-preference ratio for the corresponding control group (Fig. 1A). Otherwise, there were no significant differences ($P > 0.25$).

For turpentine, there were significant differences between groups ($F_{5,24} = 3.76$, $P < 0.01$) and periods ($F_{1,24} = 14.0$, $P < 0.02$). Because the interaction between groups and periods also was significant ($F_{5,24} = 3.3$, $P < 0.02$), the analysis was interpreted in terms of this highest-order effect. Posthoc tests showed that the mean treatment-preference ratio for the experimental group given the low turpentine concentration was significantly lower than (a) the mean pretreatment-preference ratio for this group, and (b) the mean treatment-preference ratio for the corresponding control group (Fig. 1B). Also, the mean treatment-preference ratios for experimental and control group birds given the high turpentine concentration were significantly lower than the mean pretreatment-preference ratios for these groups.

For both piperine and zingerone, there were significant differences between groups ($F_{5,24} = 3.4$, $P < 0.02$, and $F_{5,24} = 2.6$, $P < 0.05$, respectively) and periods ($F_{5,24} = 11.0$, $P < 0.003$, and $F_{5,24} = 28.9$, $P < 0.001$, respectively). In each case, mean preference ratios declined significantly as stimulus concentration increased, and mean pretreatment-preference ratios were significantly greater than mean treatment-preference ratios (Fig. 1C and 1D).

Discussion.—Our results demonstrate that European Starlings detect capsaicin in that they can be trained

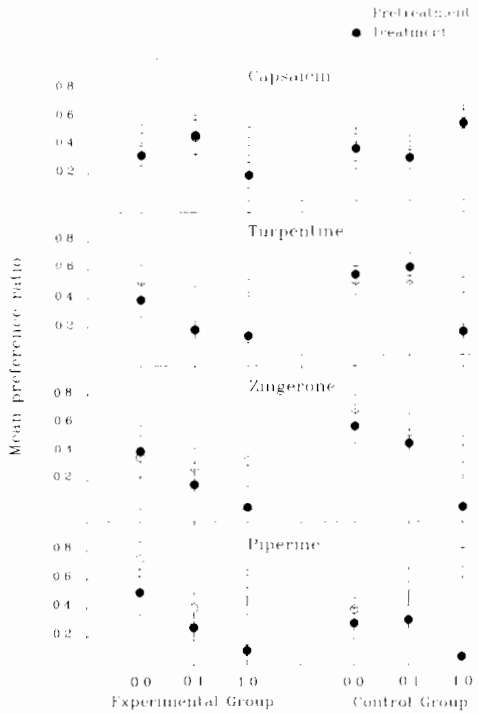

Fig. 1. Mean preference ratios (±SE) exhibited by European Starlings for capsaicin, turpentine, zingerone, and piperine.

to avoid it. However, they do not appear to perceive this mammalian irritant as an aversive stimulus. The birds were indifferent to the high concentration of capsaicin in the absence of training, and they failed to respond to the low concentration under any circumstance. These findings are consistent with several previous studies showing that naive birds fail to respond to capsaicin (Szolcsanyi et al. 1986, Norman et al. 1992).

Unlike capsaicin, the low concentration of turpentine was avoided when presentation was followed by gastrointestinal malaise, and the high concentration was avoided in the absence of training. These results suggest (a) that the birds were able to detect 0.1% turpentine, but did not perceive it as an irritant, and (b) that 1.0% turpentine was aversive. This latter result is consistent with the observation that turpentine repels Brown-headed Cowbirds (*Molothrus ater*; Mason and Bonwell 1993). Cowbirds avoid turpentine concentrations as low as 0.13% (m/m) in two-cup tests. Not all passerines avoid turpentine, however, and Red-winged Blackbirds and Common Grackles (*Quiscculus quiscula*) fail to avoid concentrations as high as 5.0% (Mason and Bonwell 1993). The reason(s) underlying this difference in sensitivity among species is unclear, although Red-winged Blackbirds and Common Grackles tend to be insensitive to a wide variety of chemical stimuli (Clark and Mason 1989, Mason et al. 1991b).

Only the high concentrations of piperine or zingerone were avoided, and the strength of responding was not dependent upon training (i.e. there were no group differences). For piperine, this result is consistent with the results of a previously reported experiment. Mason and Otis (1990) found that naive European Starlings avoid 1.0% (m/m) piperine in feed. Nevertheless, these results, coupled with the lack of response to capsaicin in the absence of training, suggest that starlings are insensitive to mammalian irritants and not simply tolerant of them.

Although birds exhibited some avoidance of the mammalian irritants tested in the present experiment, only the high concentrations were effective in the absence of training. Because mammals typically show avoidance of the low concentration (0.1% m/m) of these stimuli (Silver et al. 1985, Mason and Otis 1990), we infer that birds are both less sensitive than mammals and, at least in the case of capsaicin, that they do not perceive some compounds as irritants. The ecological rationale (if any) underlying the poor responsiveness and apparently low sensitivity of birds to mammalian irritants remains obscure. However, anecdotal evidence suggests an intriguing possibility for capsaicin. Taxonomic differences in responsiveness to capsaicin might stem from the reproductive strategy of *Capsicum* plants. Capsaicinoids may exploit the separately evolved sensory systems of birds and mammals (Mason et al. 1991c), and selectively repel mammalian seed predators, but not avian seed dispersers. These aromatic amides are present only in the red, upright fruit and occur nowhere else in *Capsicum* plants. The fruits themselves are high in vitamins, proteins, and lipids (Herrera 1987), traits that are correlated with avian dispersal (Willson and Thompson 1982, Willson and Hoppes 1986). Birds are commonly seen feeding on wild *Capsicum* fruits, colloquially known as "bird peppers." Rodents have not been observed eating these fruits, although they will readily consume *Capsicum* seeds in the absence of capsaicinoids (D. Norman pers. comm.). Whether or not the lack of response that birds exhibit to other irritants can be similarly explained remains to be determined.

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