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SHORT COMMUNICATIONS

Capsaicin detection in trained European Starlings: the importance of olfaction and trigeminal chemoreception.—Although the morphological organization of the peripheral trigeminal system in birds is not very different from that found in mammals (Dubbledam and Veenman 1978), broad functional differences exist (Kare and Mason 1986; Mason et al. 1989, 1991b; Norman et al. 1992). For example, birds rarely avoid mammalian irritants, even though the avian trigeminal system is responsive to chemical stimuli (Walker et al. 1979, Mason and Silver 1983, Mason et al. 1989). Rock Doves (*Columba livia*), Red-winged Blackbirds (*Agelaius phoeniceus*), European Starlings (*Sturnus vulgaris*), and Gray Partridges (*Perdix perdix*) are indifferent to 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 ≥1000 ppm capsaicin, the pungent principle in *Capsicum* peppers. Likewise, Red-winged Blackbirds and European Starlings are indifferent to 1000 ppm gingerol and zingerone, the irritants present in ginger (*Zingiber officinale*), as well as to 1000 ppm piperine, the active ingredient in black pepper (*Piper nigrum*) (Mason and Otis 1990). Mammals typically avoid much lower concentrations of these substances: 100 ppm capsaicin is typically avoided by rodents (Mason, pers. obs.).

The indifference that birds exhibit towards at least some mammalian irritants may reflect both insensitivity and a relatively high tolerance for these substances independent of sensation. For example, a recent series of experiments (Mason and Clark 1994) showed that starlings can detect 1.0% [mass/mass (m/m)] capsaicin in flavor avoidance learning experiments. Such learning does not occur when 0.1% (m/m) capsaicin is used as the conditional stimulus, suggesting that this lower concentration is not perceived. Both capsaicin concentrations are strongly aversive to mammals (e.g., Silver et al. 1985).

Because birds do not appear to respond to capsaicin as an irritant, the possibility exists that detection of this substance is mediated by a sensory system other than the trigeminal system. The present experiments were designed to investigate this issue. In Experiment 1, sham-operated European Starlings and starlings with bilateral olfactory nerve cuts were presented with 1.0% (m/m) capsaicin in a conditioned flavor avoidance paradigm. In Experiment 2, sham-operated starlings and birds with bilateral olfactory and trigeminal nerve cuts were similarly trained.

Methods.—Starlings were decoy-trapped in Sandusky, Ohio, and shipped by air to the Monell Chemical Senses Center. Upon arrival, the birds were individually-caged (dimensions: 61 × 36 × 41 cm) under a 13:11 h light : dark cycle (lights on 07:00–20:00 h). Prior to testing, feed (Purina Flight Bird Conditioner, Purina Mills, St. Louis, Missouri) and tapwater were provided ad libitum.

Capsaicin (CAS# 404-86-4), ethyl ether (CAS# 60-29-7), and lithium chloride (CAS# 7447-41-8) were purchased from Sigma Chemical Company (St. Louis, Mo.). For preparation of feed samples, capsaicin was dissolved in ethyl ether and then mixed with Flight Bird Conditioner to produce a concentration of 1.0% (m/m). Control feed samples were prepared by mixing ethyl ether with plain feed. All samples were placed under a ventilation hood for 48 h to evaporate the ether (Jakubas et al. 1992) and then stored at −17°C in closed containers until 30 minutes prior to use.

In the first experiment twenty-two naive starlings were assigned randomly to three groups.

The birds in group BONC (N = 8) were given bilateral olfactory nerve cuts (Mason and Silver 1983, Clark and Mason 1987, Mason et al. 1989). Briefly, birds were anesthetized by intraperitoneal injection of sodium pentobarbital (65 mg/kg body mass) and then placed in a specially designed head holder. The two olfactory nerves underlying the bony orbital walls were exposed, lifted slightly, and 1-mm sections were removed from each. The cut nerve ends were folded back to hinder regeneration, the cavity was packed with gelfoam, and the skin closed with cyanoacrylate glue. The birds in group SHAM (N = 8) were treated identically to those in group BONC, except that their olfactory nerves were not cut. The birds in group CONT (N = 6) were not subjected to surgery.

After five days of recovery from the surgical procedure, all birds were given two-cup pretreatment trials on each of four consecutive days. On each day, all birds were presented with two food cups at 08:00 h. Both cups contained 20 g of ether-treated control feed. After two hours, the feed remaining in each cup was weighed. Between 10:00 and 17:00 h EST, all birds were provided with free access to untreated feed and tapwater. Overnight (17:00–08:00 h), birds were food deprived. The food deprivation regime remained in effect throughout the remainder of the experiment.

On the day of conditioning (day 5), all birds were given a cup containing 20 g of treated feed. After two hours, the feed remaining in the cup was weighed, and the birds in groups BONC and SHAM were gavaged with lithium chloride (2 mg/kg body mass; Mason and Reidinger 1983b, Mason and Silver 1983). The birds in group CONT were gavaged with tapwater (2 mg/kg body mass).

A four-day test period immediately followed the day of conditioning. On each of these days, all birds were given two-cup tests between 08:00 and 10:00 h. One cup contained 20 g of control feed while the other contained 20 g of treated feed. As before, the weight of feed remaining in each cup was recorded at the end of the test, and the birds were provided with free access to untreated feed and tapwater until 17:00 h.

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

A three-factor analysis of variance (ANOVA) was used to evaluate the results. The independent factor in this analysis was groups (3 levels), while the repeated factors were periods (2 levels) and days (4 levels). Tukey tests (Winer 1971) were used to isolate significant differences among means ($P < 0.05$).

In the second experiment the 16 sham and BONC birds again served as subjects. The birds in group SHAM were treated as previously described. Likewise, the birds in group BONC were given bilateral trigeminal nerve cuts using procedures similar to those described in experiment 1. Briefly, each bird was anesthetized and placed in the head holder. The ophthalmic branches of the trigeminal nerve were exposed by removing the gelfoam from the cavity left by the previous surgery. The nerves were then cut, and the ends were folded back to hinder regeneration. The surgical cavity was again packed with gelfoam, and the skin was closed with cyanoacrylate glue.

After five days of recovery from the surgical procedure, all birds were given two-cup pretreatment, conditioning, and test trials identical to those described in experiment 1. At the conclusion of the experiment, preference ratios were calculated, and evaluated in a three-factor ANOVA. The independent factor in this analysis was groups (2 levels), while the repeated factors were periods (2 levels) and days (4 levels). Tukey tests were used to isolate significant differences among means ($P < 0.05$).

Results.—In experiment 1, there was a significant group × period interaction ($F = 7.1$; 2,19 df; $P < 0.01$). Post-hoc tests revealed the following pattern of effects. First, the mean

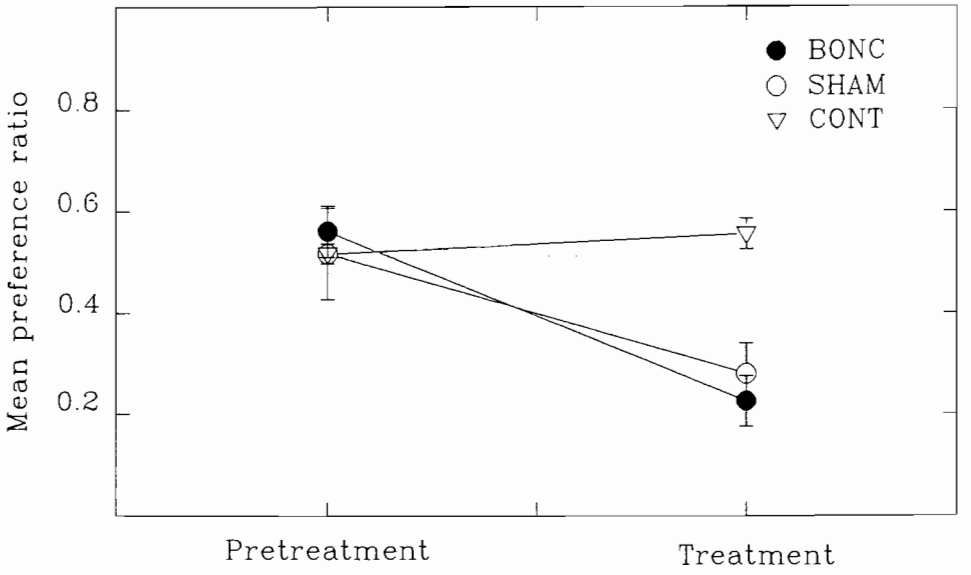

FIG. 1. Mean preference ratios exhibited by starlings given bilateral olfactory nerve cuts (BONC group) or sham surgeries (SHAM group) followed by flavor avoidance conditioining with 1.0% (m/m) capsaicin adulterated food. Control group (CONT) birds were not subjected to surgery or conditioning. Capped vertical bars represent standard errors of the means.

CONT pretreatment preference ratio did not differ from the mean CONT test preference ratio. However, both the SHAM and the BONC mean pretreatment preference ratios were significantly larger than the test preference ratios for these groups (Fig. 1).

In experiment 2, there was a significant interaction between groups and periods (F = 8.51; 1,11 df; $P < 0.01$). Post-hoc tests showed that BONC pretreatment preference ratios were significantly smaller than SHAM test period preference ratios. BONC preference ratios did not change significantly between periods (Fig. 2).

Discussion.—CONT starlings failed to avoid 1.0% (m/m) capsaicin in the absence of training. This lack of response is consistent with other reports (e.g., Mason et al. 1991a, b; Norman et al. 1992; Szolcsanyi et al. 1986), and suggests that starlings are relatively less sensitive than mammals to this chemical (e.g., Silver et al. 1985). Nevertheless, starlings can be trained to avoid 1.0% (m/m) capsaicin, providing strong evidence that this species detects capsaicin as a stimulus. The qualitative nature of this stimulus remains obscure, although the present experiments provide clues as to the sensory system mediating the behavioral response.

Olfaction appears to be unimportant. Although capsaicin has a vanillin odor to humans, bilateral olfactory nerve cuts had no measurable effect on acquisition of conditioned avoidance towards adulterated foods. In addition, the experiments were conducted during November, a time of year during which starling olfactory detection and discrimination are poor (Clark and Smeraski 1990).

Contrary to expectations, an intact trigeminal nerve was important for response acquisition. This demonstrates that starlings can detect capsaicin via trigeminal chemoreception just as mammals do (Silver et al. 1985). The surprising difference between the taxa is that the mammalian trigeminal system appears to encode capsaicin as a chemically painful stimulus, while the avian trigeminal system does not. Although the present data do not address

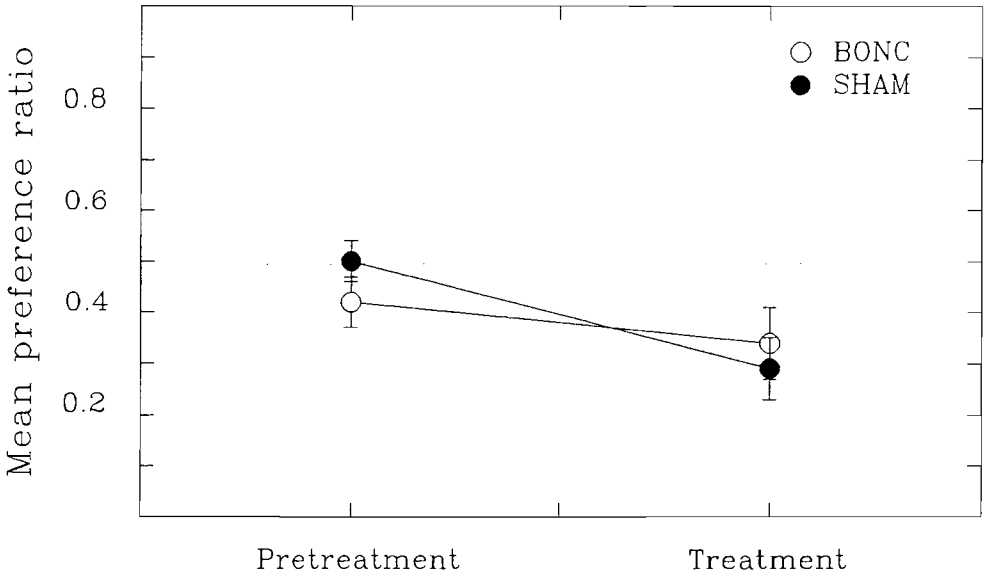

FIG. 2. Mean preference ratios exhibited by starlings given bilateral olfactory and trigeminal nerve cuts (BONC group) or sham surgeries (SHAM group) followed by flavor avoidance conditioning with 1.0% (m/m) capsaicin adulterated food. Capped vertical bars represent standard errors of the means.

the issue, we speculate that avian capsaicin detection could be mediated by thermal receptors rather than by chemoreceptors. This speculation is based on evidence that some portion of the mammalian response to capsaicin is mediated by thermoreceptors (Foster and Ramage 1981). Regardless, the results of the present investigations provide further confirmation that the chemosensory world of birds and in particular, the perception of mammalian chemical irritants, is fundamentally different from the chemosensory world of mammals.

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