

TRIGEMINAL REPELLENTS DO NOT PROMOTE CONDITIONED ODOR AVOIDANCE IN EUROPEAN STARLINGS

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ABSTRACT.—Birds, and in particular European Starlings (*Sturnus vulgaris*), avoid consumption of fluid and food treated with the natural plant products, methyl anthranilate and *o*-aminoacetophenone. Avoidance is an unlearned response most likely mediated via chemically sensitive fibers of the trigeminal nerve. The trigeminal nerve codes for chemical irritation and pain. Starlings are not repelled by the odor of the compounds, nor is olfaction important in the avoidance response. Moreover, starlings fail to learn to avoid the odor of the repellents, even after direct oral contact with liquid repellent. While trigeminal irritants can be powerful repellents, the absence of associative learning for these repellents will influence the application strategy for formulation and use. More broadly, the difference in learning abilities associated with trigeminal repellents and those commonly responsible for conditioned avoidance learning have implications for the structure of chemical defenses of fruits and the prevention of untimely frugivory. *Received 24 Feb. 1995, accepted 1 Sept. 1995.*

Nonlethal bird repellents are important components of an integrated wildlife management strategy. Repellents can be used to protect birds from human activities (Clark and Shah 1993) or to minimize damage caused by birds (Mason and Clark 1992). The social emphasis on safe, nonlethal methods to resolve conflicts between humans and birds has resulted in numerous attempts to identify new repellents (Dolbeer 1986, Crocker and Perry 1990, Clark and Shah 1991). However, reported efficacy of nonlethal repellents is highly variable (Mason and Clark 1992). In part, this is due to a misunderstanding about how repellents work. To minimize failure rates in the field, several fundamental questions about mode of action and formation of avoidance response remain to be resolved.

Nonlethal chemical repellents operate via two distinct mechanisms (Zahorik 1976), conditioned avoidance and nonlearned avoidance. In conditioned avoidance learning, birds learn to avoid sensory cues paired with a stimulus that causes illness (Garcia et al. 1966). The magnitude and persistence of the avoidance response depends on the toxic potential of the sickness producing agent and the localization of the illness. Pelchat et al. (1983) found conditioned avoidance was strongest in the rat when

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sickness was localized in the upper region of the small intestine. In birds, ingestion of carbamate insecticides (e.g., methiocarb) and fungicides (e.g., thiram, ziram) causes gastrointestinal sickness. Substances causing sickness have been used to condition birds to avoid tastes (Schuler 1983), odors (Clark and Mason 1987), and visual cues (Mason and Reidinger 1983). In a nonlearned avoidance response, substances possess taste, smell, or irritating qualities that are perceived as unpalatable by birds. Generally, in the quantities ingested, these substances do not cause sickness (Clark and Mason 1993).

Previous studies indicated that acetophenone and anthranilate bird repellents must be present in high concentrations to be effective (Clark et al. 1991). High concentrations of repellents (hundreds to thousands ppm) can be delivered orally, in food or fluid, or to the eye via aerosols. Responsiveness to only high concentrations suggests mediation by the trigeminal system as opposed to olfaction or taste (Walker et al. 1986). Chemically sensitive fibers of the trigeminal nerve mediate response to irritating and painful stimuli (Green et al. 1990). Yet coding for pain or irritation does not necessarily imply tissue damage (Clark 1995).

Common questions are whether the odors of acetophenones and anthranilates are repellent or whether birds can learn to avoid the odor of these repellents. These questions imply avoidance behavior mediated via olfaction and the formation of a conditioned avoidance response. Determining whether avoidance is influenced by olfaction or trigeminal cues is critical to the conceptualization and implementation of delivery strategies of repellents and to the understanding of how birds may respond to natural plant or insect chemical defenses.

The experiments in this paper address these question and are used to argue the point that acetophenone and anthranilate compounds are trigeminal irritants (repellents). As a test of the appropriateness of the experimental paradigm, the effect of short-term water deprivation on subsequent drinking assays was evaluated in Experiment 1. Experiment 2 tested whether naive starlings avoided the odor of a repellent and whether oral contact with a repellent was a sufficiently adverse experience to train starlings to subsequently avoid the odor of a repellent. This experiment also tested the effect of stimulus sequence on the outcome of the drinking assays. Experiment 3 tested the effect of prolonged exposure to orally delivered repellents on the subsequent response to the odor of the repellent. In Experiment 4, the role of olfaction in the mediation of the avoidance response was assessed.

METHODS

Study subjects.—European Starlings (*Sturnus vulgaris*) were decoy-trapped at Sandusky, Ohio, and transported to the laboratory in Philadelphia where they were kept in group

housing until selected for experimentation. Starlings were maintained on chick starter mash (*ad libitum*) supplemented with a vitamin mixture and fresh apples (weekly). Tap water was available continuously, except during testing. Because starlings exhibit a seasonality in their olfactory ability (Clark and Smeraski 1990), all tests were conducted during the spring when starlings have good olfactory acuity and discrimination ability. Starlings were maintained on a constant temperature (23°C), 14:10 h light:dark cycle during their residence in the laboratory.

Test stimuli.—Fluid intake for o-aminoacetophenone (OAP) and methyl anthranilate (MA) was evaluated using standard 6-h one-bottle (no-choice) drinking tests (Clark and Shah 1994). These compounds were selected as representative of nonlethal acetophenone and anthranilate bird repellents. Both compounds are natural plant metabolites that have organoleptic characteristics that humans perceive as a musky/foxy odor for OAP and a grapey odor for MA (Acree et al. 1990). Concentrations selected for testing were based upon water solubility limits of the least soluble compound (MA) and the least practical concentration yielding reliable rejection of treated fluid by most starlings.

Experiment 1: Effects of water deprivation on fluid consumption.—The objectives of the first experiment were to (1) determine the effect of diurnal water deprivation on overnight water consumption and (2) determine the effect of a three-day diurnal water deprivation schedule on post-test water intake. Determining the potential magnitude of the carry-over effect attributable to water deprivation was important for the interpretation of other experiments relating to the effects of chemical repellents. While previous studies showed that the 6-h drinking test used here is not sufficient to cause a severe water deficit in starlings (Clark and Shah 1991), it was decided that a revisitation of these questions was in order.

The experimental design consisted of a standard one-bottle, 6-h assay. On the first day (the pre-test period), 18 birds were randomly assigned to three groups ($N = 6/\text{group}$) and presented with tap water in graduated Richter tubes. The recording period began at 10:00 and ended at 16:00 (hereafter defined as the diurnal test period). The Richter tubes were replaced with a second set of tubes and water was available *ad libitum* through the period 16:00 to lights out at 19:00, throughout the night, and for the period from lights on at 7:00 to 10:00, the start of the next test sequence (hereafter defined as the overnight period). As a precondition for further testing, similarity for average diurnal water consumption among groups was verified using a one-way fixed effects analysis of variance (anova).

On the second day, birds within the groups were presented with one of three randomly assigned treatments. Birds within the control group were presented with tap water. Birds within the second group were presented with a 28 mM solution of o-aminoacetophenone (OAP). Because OAP is a potent bird repellent, it was anticipated that this group would experience voluntary water deprivation by avoiding the treated water (Clark and Shah 1991). Birds within the third group were water deprived by physically excluding them from the Richter tubes. The protocol for recording water consumption for the diurnal and overnight periods followed that described above. Birds retained their water presentation treatment assignments for days three and four, and water intake for the diurnal and overnight periods followed the format described above.

On the fifth day (the post-test period), birds within all three treatment groups were presented with untreated tap water and intake was monitored according to the prescribed protocol.

The first question, "... does diurnal fluid deprivation affect overnight water consumption?", was addressed using a 3×3 repeated measures fixed-effect analysis of covariance (ancova). The dependent variable was overnight water consumption. The previous day's diurnal fluid intake was used as a covariate. Because the covariate was measured each day along with the dependent variable, it was treated as a changing covariate, i.e. separate

residuals were calculated for each day. Days were treated as a repeated measure, and group was treated as a between measures effect. Multivariate criteria (Rao R, Wilk's lambda) were used to simultaneously test repeated measures contrasts on adjusted means when there were more than two levels on the repeated measure. This approach is to be preferred because it does not rely on the assumption that the repeated measures are independent. In circumstances where only univariate statistics were estimable, i.e., one degree of freedom, the *F* statistic was used to assess significance. The latter case was most common in planned comparison of treatment levels by contrasts. All post-hoc tests were analyzed using a Scheffe's test. This and subsequent analyses were carried out using the STATISTICA® (1994) software packages (MANOVA procedures). This software is particularly suited to repeated measures and other longitudinal designs.

The second question, "... did the intervening treatments affect post-test water intake relative to the pretreatment period?" was addressed using a 2×3 repeated measures fixed-effect ANOVA. The dependent variable was diurnal fluid intake, period (pre vs post test) was a within measures (repeated) effect, and group (i.e., water deprivation schedule) was a between measures effect.

Experiment 2: Effects of odor and treatment sequence on water consumption.—Starlings readily form conditioned odor avoidance responses when the unconditional stimulus produces a strong gastrointestinal illness (Clark and Mason 1987). Starlings also avoid some substances upon initial contact, indicating that the avoidance is not learned (Clark and Mason 1993). This latter response indicates a different mechanism for avoidance, one that suggests hedonic attributes (e.g., taste, smell, irritation) form the basis of acceptance or rejection. Clark and Mason termed these compounds sensory repellents. The objectives of this experiment were to (1) determine whether oral and/or nasal exposure to OAP influenced fluid intake, (2) determine whether starlings could learn to avoid the odor of OAP once they had an aversive oral exposure to the substance, and (3) assess whether the order of presentation of OAP in solution or odor influenced the behavioral response.

Thirty-six starlings were randomly assigned to six groups of six birds each. On the first day, birds were tested for baseline water consumption in a standard 6-h drinking test following procedures outlined in Experiment 1. Attached to both sides of the Richter tube sipping port was a 40 mm diameter, opaquely screened polypropylene disk containing a wick saturated with tap water.

During the three-day test period, birds were presented with one of three treatment conditions (Richter tube/wick disk combinations): [w/w] a tap water filled Richter tube and a tap water soaked wick, [o/w] a Richter tube filled with 28 mM OAP solution and tap water soaked wick, and [w/o] a tap water filled Richter tube and an OAP soaked wick. Presentations of the Richter tube/wick combinations to experimental cohorts were established according to two possible Latin square designs (Table 1). On the fifth day (post-treatment period), all birds were presented with the control tube/wick pairing (w/w). Diurnal and overnight fluid intake were monitored following procedures outlined in Experiment 1.

Data were analyzed using a fixed-effects $3 \times 3 \times 6$ nested ANOVA design with repeated measures. Diurnal fluid intake was the dependent variable and day was a repeated measure with three levels. Sequence (two levels) and group (six levels) were between measures effects, with group nested within sequence (Table 1). To test whether the context of stimulus presentation is important for repellency, contrasts between w/w and o/w, and w/w and w/o were made for the first day of testing. These comparisons addressed the question of whether OAP in solution (where olfactory, gustatory, and trigeminal systems may influence responding) or whether OAP as an odor stimulus (where olfactory and trigeminal systems may influence responding) were important in the formation of an avoidance response. By considering only the first day of testing, these comparisons controlled for possible learning

TABLE 1
LATINIZED DESIGNS FOR THE PRESENTATION ORDER OF TREATMENTS^a

	Design 1				Design 2		
	Day	1	2		3	Day	1
Group 1	w/w	o/w	w/o	Group 4	w/o	o/w	w/w
2	o/w	w/o	w/w	5	o/w	w/w	w/o
3	w/o	w/w	o/w	6	w/w	w/o	o/w

^a Experimental codes are: (w/w) tap water presented in the Richter tube with blank odor disks on either side of the drinking port; (o/w) 28 mM OAP solution in the Richter tube with blank odor disks on either side of the drinking port; (w/o) water presented in the Richter tube with saturated OAP wicks in the odor disks on either side of the drinking port.

effects while considering whether repellency is mediated via nasal/ocular (i.e., volatile) or oral/nasal/ocular (i.e., contact and volatile) mechanisms. Similar contrast analyses comparing w/w to w/o were performed for days two and three. These tests allowed for the possibility of learning while addressing the question whether consumption for birds exposed to odor differed from that of the control condition.

OAP is a good sensory repellent when delivered orally. However, can oral presentation of OAP be used to condition birds to avoid the odor of OAP? This question was addressed by inspection of contrasts of the day \times treatment interaction term. Intake for birds presented with treatment levels w/w and w/o on day 2 were compared for those birds presented with o/w on day 1, i.e., the putative training day. The overall sequence effect throughout the test was determined by consideration of the main effect and by inspection of post hoc differences among means grouped by treatment category using the Scheffe's test.

Experiment 3: The conditioned odor avoidance response.—In the absence of water deprivation effects (Experiment 1), sequence effects (Experiment 2), and an apparent inability for short exposure to orally delivered OAP to act as an unconditional stimulus (Experiment 2), the length of time starlings were orally exposed to a chemical repellent was increased to determine whether a conditioned avoidance response towards odor could be attained.

Separate experiments on two bird repellents were carried out, and the experimental design of these tests was as follows. Starlings were tested serially for fluid intake in a standard no-choice (one-bottle) 6-h drinking assay. On the first day, eighteen starlings were drawn at random from the group housing pool, randomly assigned to one of two groups (N = 9/group) and pre-test water consumption was monitored according to methods described in Experiment 1. Attached to both sides of the Richter tube sipping port was a 40 mm diameter, opaquely screened polypropylene disk containing a wick saturated with tap water (w/w), following the nomenclature convention in Experiment 2).

For each of three successive days (2–4), starlings within groups were presented with Richter tubes containing a 28 mM solution of a sensory repellent (OAP or MA) and diurnal fluid intake was monitored as described above (Experiment 1). Attached to both sides of the Richter tube sipping port was a wick saturated with tap water (o/w). The strong odor derived from the repellent emanated from the drinking port of the Richter tube, i.e., repellent solution.

On the fifth and sixth day, starlings were presented with Richter tubes containing tap water and fluid intake was monitored as above. During this period the wick inside the disks was saturated with repellent (w/o). Thus, the strong odor of the repellent was present in the apparatus but did not originate from the solution.

Data were analyzed using a fixed effects one-way ANOVA with repeated measures on

days/treatment effect. The confounding of day and treatment on water intake was considered unimportant. Thus, in the absence of carryover effects due to diurnal water deprivation (Clark and Shah 1991, Experiment 1), any day/treatment effect was to be interpreted primarily as a treatment effect. In addition to the day/treatment effect, a single *a priori* planned comparison was made using contrasts. The w/w condition on day 1 was compared to the w/o condition (days 5–6) as an assessment for the formation of a conditioned odor avoidance response. The results for the OAP and MA tests were analyzed separately.

Experiment 4: The role of olfaction in the avoidance response.—Odors can stimulate the olfactory system, but they can also gain access to chemically sensitive receptors of the trigeminal system via the mouth, nose, or eye. Thus, avoidance of an odor might be based upon its unpleasant smell or on its irritating/painful properties.

The object of Experiment 4 was to determine the influence of olfaction on fluid intake when odor was present in the fluid to be consumed. The experimental design was as follows. Starlings were tested for fluid intake in a standard no-choice (one-bottle) six hour drinking assay. The experiment comprised two surgical conditions (Surgery effect: sham or bilateral olfactory nerve cut [BONC]), two repellent concentration conditions (Concentration/Group effect: 14 mM and 28 mM), and two test periods (day/treatment effect: pretreatment = water presentation and treatment = chemical repellent presentation). The confounding of time and treatment was considered to be unimportant (Experiment 1), and any differences were assumed to be attributable to the presence of repellent. Data were analyzed using a fixed effects, 3-way analysis of variance with repeated measures on the day/treatment effect.

Sixteen starlings were selected at random from the group housing pool and randomly assigned so that eight received a BONC and the other eight received a sham surgery. Starlings were anesthetized with choral hydrate/penobarbital at a dose of 2 ml/kg, intraperitoneally and placed in a head-holder. Surgery for BONC and sham treatments followed procedures outlined in Clark and Mason (1987). Following surgery, starlings were housed individually in cages (61 × 36 × 41 cm). Two weeks following surgery, birds were adapted to experimental conditions. The two-week latency period was estimated to provide sufficient time for degeneration of olfactory afferents into the olfactory bulb, yet not sufficiently long to allow olfactory nerve regeneration (Wenzel and Salzman 1968). Following adaptation one half of the birds within the BONC and sham surgery treatments was randomly assigned to concentration groups, resulting in four birds per experimental cell. On the first day of testing (pretreatment period), beginning at 09:30 h, consumption of tap water was recorded every 2 h for the next 6 h. On the second day, starlings were presented with their preassigned concentration of OAP and fluid intake was monitored every 2 h for the next 6 h. Fluid was presented in 120 ml graduated Richter tubes. Starlings were visually isolated from one another as well as from the contents of the Richter tubes. After five days rest, the same birds used in the above experiment were re-randomized with respect to concentration group assignment and tested with MA using the above protocol.

At the end of the experiments, birds were killed with pentobarbital and sequentially perfused with physiological saline, 10% formalin and a 10% formalin and 30% sucrose mixture. Brains were removed and the effect of the nerve section on olfactory bulb structure was evaluated in a double blind sequence. Briefly, the anterior tips of the hemispheres (containing the olfactory bulbs) were cut into 50 μ m slices. Every fifth slice was stained with Nissl stain to highlight the outline of the glomeruli. The observer (familiar with normal histological structure of avian olfactory bulbs) categorized the coded histological series as being either in a degenerative state or normal. Concordance of the observer's scoring and the surgical status were compared using a chi-square analysis. Degeneration of the glomerular structure was taken as evidence for lack of olfactory nerve input into the olfactory bulb (Wenzel and Salzman 1968).

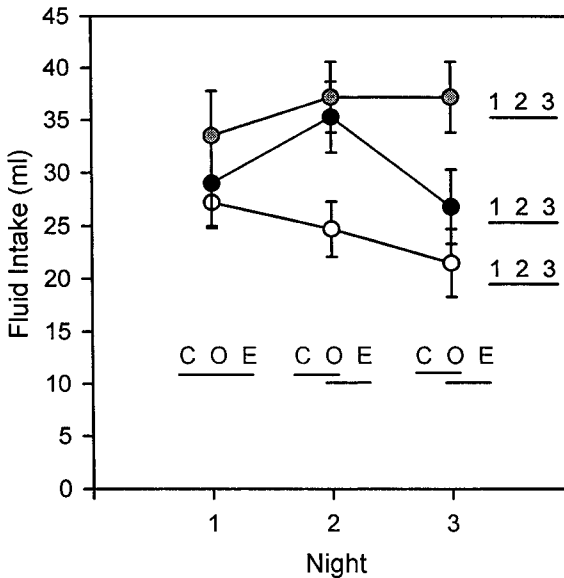


FIG. 1. Average overnight water consumption as a function of time and water deprivation schedule (Experiment 1). Open circles = control group (C); solid circles = OAP group (O); Shaded circles = water excluded group (E). Horizontal bars depict statistically similar water consumption (Scheffe's test, $P > 0.05$). Letters compare average intake across treatment categories within a single night. Numerals compare average intake across nights within a single treatment category. Vertical capped bars depict \pm one standard error.

Zinc sulfate ($ZnSO_4$) also has been used to render birds anosmic. However, because $ZnSO_4$ strips away the olfactory epithelium (Burd 1993), it may also affect epithelial layers containing trigeminal free nerve endings. There are no data on this latter point. Because of this uncertainty, surgical manipulation of the olfactory nerve was deemed to be the best way to determine the role of olfaction in the avoidance response.

Directly eliminating trigeminal input is not feasible. In birds, only the ophthalmic branch of the trigeminal nerve (OBTN) is easily accessible for denervation studies (Getty 1975). Manipulation of the maxillary and mandibular branches would require too much destruction of bone and muscle tissue. All three branches could be eliminated at the Gasserian ganglion located in the eye orbit, but this would require permanent blinding of the bird.

RESULTS

Experiment 1: Effects of water deprivation on fluid consumption.—The overnight fluid intake for the treatment groups, adjusted for the covariate of diurnal fluid intake, differed across days (Rao $r = 4.55$, $P < 0.007$). The post-hoc analysis indicated that the overnight water consumption within treatment groups was similar across nights (Fig. 1). However, water consumption differed among treatments within nights. On the first night, all birds consumed similar amounts of water. On the second and third

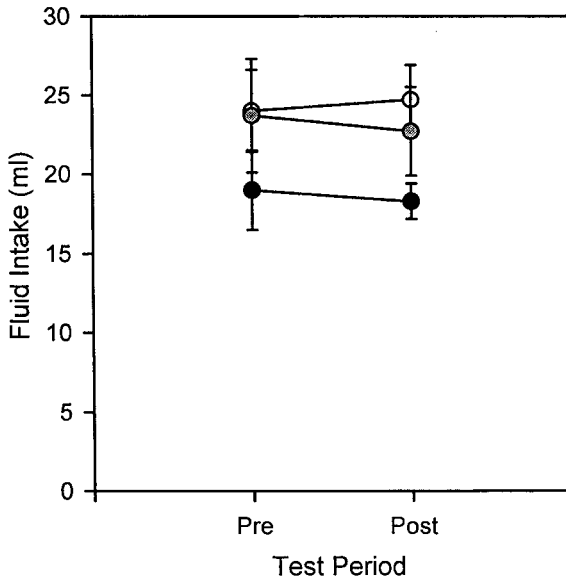


FIG. 2. Average diurnal water consumption as a function of treatment group and time (Experiment 1). Open circles = control group; solid circles = OAP group; shaded circles = water excluded group). Vertical capped bars depict \pm one standard error.

nights the control birds drank less water than the birds that were physically excluded from water during the day. Overnight consumption for birds presented with OAP did not differ from the other two experimental groups. There was an inverse, albeit nonsignificant ($P < 0.099$), relationship between daytime fluid intake and subsequent overnight water consumption (Fig. 1). Birds experiencing fluid deprivation by exclusion tended to have the highest overnight water intake ($36.0 \text{ ml} \pm 3.7 \text{ SE}$), followed by birds experiencing deprivation by repellency ($30.4 \text{ ml} \pm 3.6 \text{ SE}$). Control birds that had *ad libitum* access to water showed the lowest overnight water consumption ($24.3 \text{ ml} \pm 2.7 \text{ SE}$). Treatment category did not appear to affect baseline diurnal water consumption immediately following the tests (Fig. 2). There were no group ($P < 0.453$), test period ($P < 0.889$), or interaction ($P < 0.362$) effects.

Experiment 2.—The context of the stimulus is important for repellency. With no prior experience, starlings presented with OAP in solution consumed less fluid than the controls ($F = 94.86$, $df = 1,30$, $P < 0.001$). This observation stands in contrast to the similarity of diurnal fluid intake for the controls (w/w) and birds presented with water and OAP odor (w/o) ($P < 0.45$). Thus, odor alone is not a sufficiently strong stimulus to

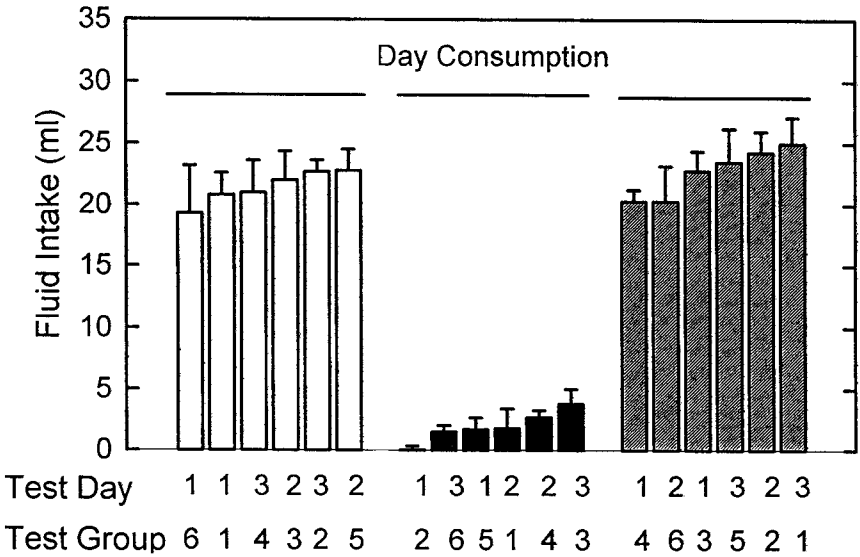


FIG. 3. Average, diurnal fluid intake as a function of time and treatment (Experiment 2). Birds in the control group (w/w, open bars) were presented with tap water in the drinking tubes and disks contained water soaked wicks, birds in the OAP group (o/w, solid bars) were presented with a 28 mM solution of o-aminoacetophenone in the drinking tubes and disks contained water soaked wicks, birds in the odor group (w/o, hatched bars) were presented with water filled drinking tubes and wick soaked in OAP. Average intake is ranked within treatment type and nonsignificant ($P > 0.05$) differences among means are depicted by the horizontal bar. Test Day and Test Group are the column and row label designations of the two Latinized presentation designs described in Table 1. Vertical capped bars depict one standard error.

result in avoidance. This pattern persists even for experienced birds. On days two and three the comparison w/w to w/o indicates similar levels of fluid intake ($P < 0.966$ and 0.21 , respectively), irrespective of the previous treatment exposure. The lack of an overall test sequence ($P < 0.305$) or day ($P < 0.121$) effect on diurnal fluid intake is apparent in Fig. 3.

Starlings do not readily acquire a conditioned avoidance response to the odor of OAP when orally delivered OAP is used as the unconditional stimulus. Comparison by contrasts of w/w to w/o on the second day of testing when both groups received o/w on the first day failed to uncover differences in fluid consumption ($P < 0.591$).

As was the case for Experiment 1, the intervening three day treatment schedule had no effect on baseline water consumption. There were no group, day or interaction effects for the pre-test vs post-test comparison

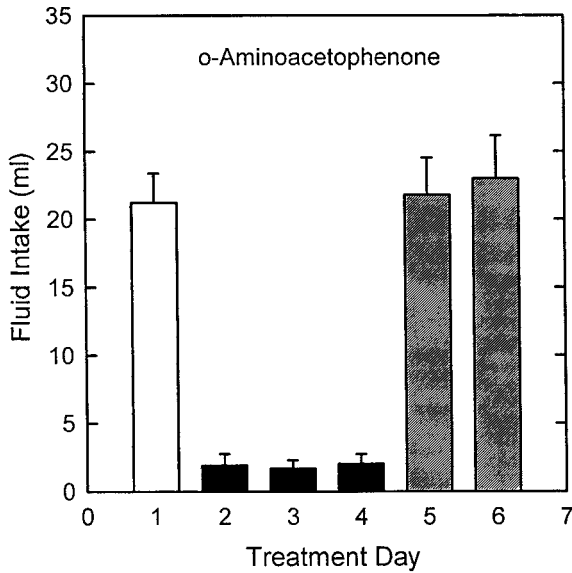


FIG. 4. Average fluid intake for OAP as a function of time and solution treatment (Experiment 3). The left most open bar depicts intake of tap water paired with a water soaked wick in the odor disc (w/w). The middle solid bars depict fluid intake of a 28 mM o-aminoacetophenone (OAP) solution paired with a water soaked wick in the odor disc (o/w). The right most hatched bars depict fluid intake of tap water paired with an OAP soaked wick in the odor disc (w/o). Vertical capped bars depict one standard error.

of diurnal water consumption ($P < 0.351$, < 0.434 , < 0.674 , respectively).

Experiment 3: The conditioned odor avoidance response.—There were significant day/treatment effects for both the OAP and MA tests (Rao's $r = 9.56$, $P < 0.024$ and $r = 12.48$, $P < 0.15$, respectively). The planned comparisons indicated that fluid consumption was similar for the w/w and w/o treatment conditions for both OAP ($P < 0.72$, Fig. 4) and MA ($P < 0.87$, Fig. 5). Thus, three days exposure to orally administered repellent was not sufficient for the formation of a conditioned avoidance response to that odor.

Experiment 4: The role of olfaction. There was a strong day/treatment effect for OAP ($F = 74.57$, $df = 1,12$, $P < 0.001$), showing that overall fluid intake was suppressed when the starlings were presented with either 28 or 14 mM OAP solutions (Fig. 6). None of the other effects achieved probability levels less than $P = 0.15$. The planned comparisons between sham-OAP and BONC-OAP within each concentration showed that surgery had no effect on avoidance of OAP ($P < 0.619$, 0.62 , respectively).

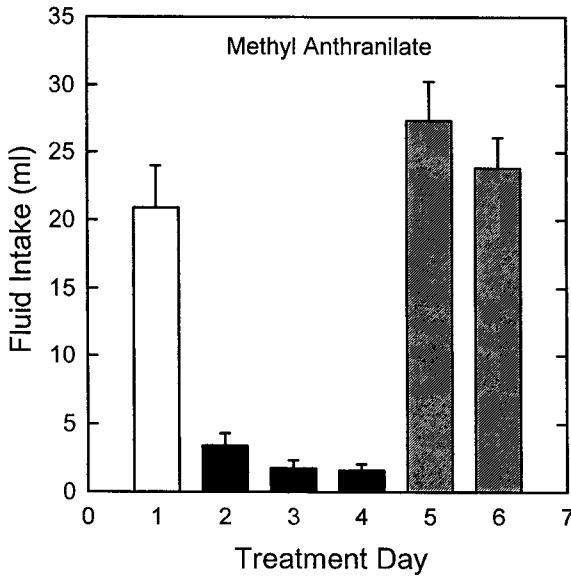


FIG. 5. Average fluid intake for MA as a function of time and solution treatment (Experiment 3). The left most open bar depicts intake of tap water paired with a water soaked wick in the odor disc (w/w). The middle solid bars depict fluid intake of a 28 mM methyl anthranilate (MA) solution paired with a water soaked wick in the odor disc (o/w). The right most hatched bars depicted fluid intake of tap water paired with a MA soaked wick in the odor disc (w/o). Vertical capped bars depict one standard error.

Similar consumption patterns were observed for the MA trials. There was a strong period effect for MA ($F = 83.14$, $df = 1,12$, $P < 0.001$), showing that fluid intake was suppressed across all surgery and concentration levels when starlings were presented with MA solutions (Fig. 7). No other effect achieved probability levels less than $P = 0.22$. The planned comparisons between sham-MA and BONC-MA within each concentration showed that surgery had no effect on avoidance of MA ($P < 0.215$, 0.399 , respectively).

Post-mortem visual inspection of the glomerular layer of sham surgery birds showed a diffuse pattern seen in normal starlings, typical for the spring breeding season. In contrast, BONC birds showed a complete break down of the glomerular structure. The birds were euthanized 20–25 days after surgery. The observer scoring the slides, but blind to the experimental identity, categorized all BONC tissue as having degenerative glomeruli ($N = 8$) and all SHAM tissue as being normal ($N = 8$) ($\chi^2 = 16.0$, $df = 1$, $P < 0.001$).

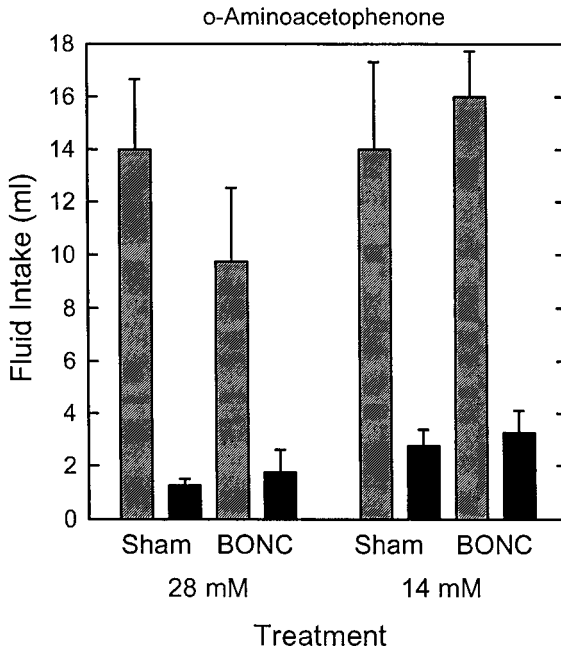


FIG. 6. Average fluid intake for OAP as a function of a solution's molar concentration, surgery and treatment period. Starlings were presented with tap water during the pretreatment period (hatched bars) and an OAP solution during the treatment period (solid bars). Capped vertical bars depict one standard error.

DISCUSSION

When deprived of water during the day, starlings drank more water than normal during the overnight recovery period. However, there was no difference between pre- and post-test diurnal water consumption, irrespective of the experimental water deprivation schedule (Experiment 1). Together these data suggest that the effects of short-term water deprivation are ameliorated during the 18-h recovery period. This lack of carryover, i.e., day effect, is consistent with previously reported results for similar experiments (Clark and Shah 1991).

The use of the two Latinized square treatment presentation sequences allowed a detailed analysis of the influence of delivery route on the avoidance response (Experiment 2). Naive starlings that were exposed to only the odor of OAP did not avoid the drinking apparatus, suggesting that the odor of OAP was not repellent. Only when OAP was allowed to come in contact with the mouth did starlings show an avoidance response.

Starlings failed to avoid the odor of OAP even after they had come in

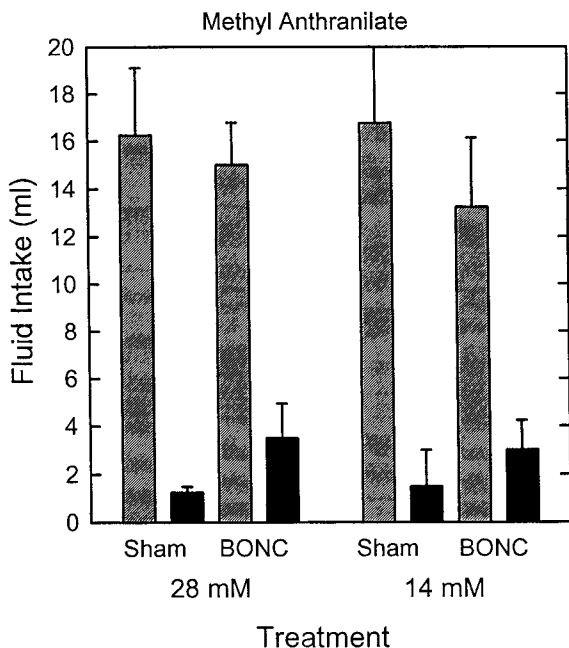


FIG. 7. Average fluid intake for MA as a function of a solution's molar concentration, surgery, and treatment period. Starlings were presented with tap water during the pretreatment period (hatched bars) and a MA solution during the treatment period (solid bars). Capped vertical bars depict one standard error.

oral contact with the substance (Experiment 2). Moreover, the prolonged exposure to repellents (OAP and MA) over several days failed to result in a learned odor avoidance (Experiment 3). These findings suggest that starlings do not readily learn to avoid solutions associated with the odor of repellents. The failure to form a conditioned avoidance is independent of the history of exposure to odors or repellent solutions (Experiment 2). Experiment 4 showed the lack of importance of olfactory cues in avoiding repellents. Surgical elimination of the olfactory nerves did not cause birds to increase consumption of repellent-bearing solution as was expected if olfaction was the mediating mechanism for avoidance.

The lack of odor avoidance and contribution of the olfactory system in the avoidance response is consistent with previous speculation that sensory repellents are mediated by the trigeminal system, although the role of taste cannot be ruled out on the basis of these experiments alone. However, there is other evidence consistent with involvement of the trigeminal nerve for perception of OAP and MA. Mason et al. (1989)

showed that bilateral section of the OBTN virtually eliminated the avoidance response of starlings to anthranilates. There is also electrophysiological evidence that the OBTN is highly responsive to OAP and MA (Clark, unpubl. data). Molecular modelling evidence suggests birds have a receptor mechanism that is responsive to anthranilates and acetophenones and is analogous to the vanilloid pain receptors in mammals (Clark and Shah 1994). Finally, even after central taste nuclei are ablated, chickens continue to avoid MA (Benowitz 1964), suggesting that taste is not critical in mediating the avoidance response.

The unsuitability of oral exposure to repellents as the unconditional stimulus may be attributable to its mode of action and/or localization. The trigeminal repellents tested appear to be unpalatable but do not cause illness (at least for the quantities voluntarily consumed by birds). In addition, the aversive effect is localized in the mouth (in this experiment) and not in the gastrointestinal system. Thus, repellents that are unpalatable and cause oral stimulation, in combination or alone, may be ineffective as unconditional stimuli relative to repellents that cause illness and gastrointestinal stimulation. This does not suggest that trigeminal repellents are poorer repellents than associative repellents. Both can be potent and result in strong avoidance behavior. The difference between the two repellent types is that birds fail to learn from their aversive experience with trigeminal repellents.

From a practical viewpoint, failure to learn about the sensory attributes of a repellent is not crippling to the objective of bird repellency. Laboratory and field studies show birds continuously sample small quantities of substances treated with sensory repellents (e.g., Mason et al. 1985, Clark and Shah 1991, 1994; Avery and Decker 1994). While total amounts consumed are small, how does the trigeminal repellent work to repel birds away from an area? The effectiveness of the repellent is two-fold. In the first case, the repellent has a direct effect on individuals. When movement is not restricted, a bird encountering a sensory repellent quickly moves on to a more palatable resource patch (Mason et al. 1985). This observation is consistent with optimal foraging theory (Charnov 1976) and the proposition that low rates of energy return in a patch will favor the probability of abandoning that patch (Lima 1985). The repellent can be viewed as signalling low energy return or physically achieving the fact. Regardless, the outcome is the same. Second, as a consequence of reduced residency time in a patch, the recruitment opportunities to that patch are reduced. Thus, the number of birds that might visit the patch because they observe other individuals in the patch is reduced (Krebs 1974).

The inability to learn to stay away from sensory cues associated with

trigeminal repellents has implications for the evolution of plant-bird interactions as well. The behavior for free-ranging birds is to sample fruit throughout its development, apparently giving more attention to the salient chemical cues associated with palatability rather than ancillary sensory cues (Willson and Comet 1993). To prevent untimely frugivory, it is to the plant's advantage to employ transient protection to fruits and have birds return to the fruits at a later time when the seeds are ready for dispersal, rather than have birds form a strong conditioned avoidance response (e.g., Brower 1969, Guarino et al. 1974, Mason 1989). These observations provide intriguing interpretive possibilities about how chemical defenses of fruit should be structured to exploit the sensory systems and learning capabilities of birds. Chemical defenses in unripe bird dispersed fruit should consist of trigeminal repellents and not compounds that cause gastro-intestinal illness. Finally, a better understanding of how plants prevent untimely frugivory will prove invaluable in the design of nonlethal repellents for safe wildlife management strategies.

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