



An exception to the rule: common vampire bats do not learn taste aversions

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Conditioned taste aversions function by preventing an organism from ingesting a food previously associated with gastrointestinal malaise. Taste-aversion learning has been observed in many animals: molluscs to mammals, insects to birds. However, among mammals, neither bats nor monophagous species have been investigated adequately. Here we show that although three dietary generalists (one insectivorous and two frugivorous bats) readily acquired taste aversions, the common vampire bat, *Desmodus rotundus*, a monophageous feeder on vertebrate blood, did not learn to associate a novel flavour with aversive gastrointestinal events. We interpret these data as consistent with the hypothesis that taste aversions are an adaptive specialization of learning.

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To cope with nutritional demands, energy requirements and toxins, animals have evolved behaviours that enable them to choose foods that are beneficial and avoid repeated ingestion of those that are unsafe (Rozin & Kalat 1971; Bures et al. 1998). Such avoidance of toxic food items results from both innate and learned behaviours (Speed 2000). For example, the coloration and pattern of coral snakes, *Micrurus fulvius*, is recognized without prior exposure by domestic chicks, *Gallus gallus domesticus* (Schuler & Hesse 1985). Other toxic food items are not so deadly as coral snakes, and an individual can learn to avoid ingesting them (Staddon 1983).

Taste-aversion learning results in an organism avoiding repeated ingestion of a food that has previously been associated with gastrointestinal malaise (Rozin & Kalat 1971), and is thought to have evolved as a defence against repeated ingestion of naturally occurring toxins (Revusky & Bedarf 1967; Rozin & Kalat 1971; Garcia et al. 1976). Taste-aversion learning has been described as an adaptive specialization of learning. Animals are considered to be uniquely predisposed to make associations between ingested flavours and symptoms of digestive poisonings rather than using visual or auditory cues (Garcia et al. 1966; Seligman 1970; Rozin & Kalat 1971). To our

knowledge, all mammals tested to date under controlled conditions learn to associate experience of novel flavours with subsequent illness in a single trial, even with considerable delay between tasting and toxicosis. However, no direct evidence supports this generally accepted explanation that the unique features of taste-aversion learning are adaptive specializations rather than a product of more general processes.

In the natural environment, taste aversions should reduce the likelihood of future poisonings and, particularly when associated with aposematic cues, should increase an animal's foraging efficiency (Brower et al. 1968; Nicolaus et al. 1983). The vast majority of microchiropteran bats, whether they eat animals or plants, should often encounter toxic food items while foraging (i.e. arthropods, Eisner 1970; frogs, Ryan & Tuttle 1983; plants, Cipollini & Levey 1997). However, taste-aversion learning has not been adequately investigated in this suborder. The purpose of this study was to determine the incidence of taste-aversion learning to novel flavours in three generalist species of microchiropteran bat and to compare their behaviour with that of a blood-feeding specialist. The generalist species were the big brown bat, *Eptesicus fuscus*, a 15–20-g insectivore; the Antillean fruit-eating bat, *Brachyphylla cavernarum*, a 35–50-g primarily frugivorous species (although anecdotal evidence suggest this species also consumes both nectar and small invertebrates); and the Jamaican fruit bat, *Artibeus jamaicensis*, a 30–45-g frugivore. The specialist was the common vampire bat, *Desmodus rotundus*.

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We selected subject species for comparative purposes based on phylogenies constructed from total evidence and morphological analyses (McDaniel 1976; Wetterer et al. 2000). Although the evolutionary relationships among the phyllostomids *A. jamaicensis*, *B. cavernarum* and *D. rotundus* are controversial (Wetterer et al. 2000), *B. cavernarum* appears to be the closest extant relative of the vampire clade (McDaniel 1976; Wetterer et al. 2000). *Eptesicus fuscus* is a member of the family Vespertilionidae, and served as an outgroup in this study.

Over evolutionary time, the anatomy and behaviour of common vampire bats and their immediate sister taxa, *Diaemus youngii* and *Diphylla eucadata*, have become highly specialized for feeding solely on blood of live animals (Fenton 1992), a food which is unlikely ever to be toxic to these species. Without exposure to potentially toxic foods, neural substrates in *D. rotundus* that supported taste-aversion learning should not have been maintained by stabilizing selection and may have deteriorated (Rozin 1976; Daly et al. 1982). Thus, we hypothesized that generalist bats should readily acquire taste aversions, while *D. rotundus*, unlike other mammals and birds studied to date (Garcia et al. 1976; Bures et al. 1998) should not be predisposed to make taste-toxicosis associations.

GENERAL METHODS

Sources of Subject Species

We conducted experiments using *A. jamaicensis* and *E. fuscus* as subjects from March to August 2000 at York University (Toronto, Canada). We removed *E. fuscus* from a private residence at the request of its owner. *Artibeus jamaicensis* was obtained from a mixed-species colony kept under seminaturalistic conditions at the Bio-dome (Montreal, Quebec, Canada). Both species resided in the laboratory in wooden boxes, measuring 70 × 50 × 30 cm. To allow perches for roosting, the sides, back and roof of the interior of each box was ridged. The front was a Plexiglas panel.

We maintained *E. fuscus* on a 16:8 h low light:dark (LLD) cycle, which was roughly the natural light schedule at the time of capture. *Artibeus jamaicensis* was kept on an LLD 12:12 h cycle, which was the light schedule used at the Bio-dome and similar to that of areas in which *A. jamaicensis* is found. We captured and tested *B. cavernarum* on Montserrat, caught *D. rotundus* near Cali, Colombia, and tested them in Palmira, Colombia, using the facilities of the Instituto Colombiano Agropecuario (ICA). After capture and throughout testing members of both species resided in cylindrical cages (30 cm high and 23 cm in diameter) made of ungalvanized hardware cloth. Both species were kept on an LLD 12:12 h cycle.

All animals, save *A. jamaicensis*, were released near their point of capture when experiments were completed. No wild-caught species was in captivity longer than 28 days. *Artibeus jamaicensis* subjects are currently being used in unrelated behavioural experiments.

Feeding bouts for all species commenced, on average, 1.5 h after the onset of the dark cycle, and water was provided ad libitum. In our study, as has been observed in the past (Greenhall & Schmidt 1988), *D. rotundus* did not drink the water provided.

EXPERIMENT 1

The importance of novelty and saliency to flavour cues in taste-aversion learning has been outlined in previous studies (see Rozin & Kalat 1971 for a review). Citric acid (sour) is the sole flavorant tested to which *D. rotundus* has shown reliable behavioural response (Thompson et al. 1982). Our purpose was to determine what concentrations of this novel flavorant *D. rotundus* could detect in defibrinated bovine blood (see also Suthers 1970; Thompson et al. 1982).

Methods

Subjects

Twelve *D. rotundus* served as subjects in experiment 1. These animals did not serve as subjects in experiment 2.

Apparatus

We housed all subjects together in a large cage, measuring 3 × 2 × 2 m.

Procedure

We provided subjects with blood for 1.5 h/day for 14 consecutive days. On odd days, we measured the group's intake of unflavoured blood. On even days, we determined their intake of blood adulterated with decreasing concentrations of citric acid.

Results and Discussion

On day 10, when we offered vampire bats blood flavoured with 0.4% weight/volume citric acid, they ingested less than on either day 9 or day 11 when we offered them unflavoured blood (Fig. 1). We used a concentration of 0.4 g/100 ml of citric acid as a conditional stimulus in experiment 2. We chose this concentration of citric acid for use in experiment 2, which was a study of taste-aversion learning, because it both produced a detectable decrement in blood intake (indicating that the presence of citric acid was detected) and left sufficient intake to avoid floor effects in control groups.

EXPERIMENT 2

To determine whether these four species of bats learn taste-aversions, we used experiments of the same general design to examine taste-aversion learning in the three dietary generalists and in *D. rotundus*.

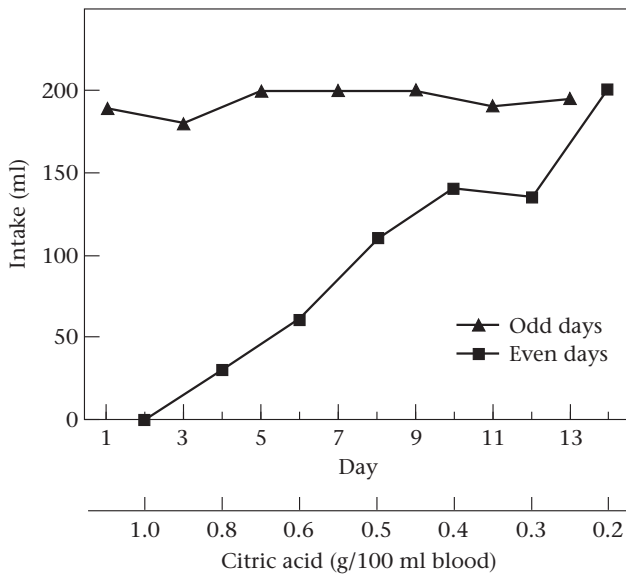


Figure 1. Mean amount of blood (odd days) or blood flavoured with decreasing concentrations of citric acid (even days) ingested by the 12 *D. rotundus* subjects in experiment 1.

Methods

Subjects

We examined 18–24 bats of each species.

Apparatus

We tested each subject in its home cage.

Procedure

To begin, we placed all subjects in the experimental situation and offered them a standard food for 1 h/day. *Desmodus rotundus* (an additional 23 animals, captured from the same cave) were fed unflavoured defibrinated bovine blood. *Artibeus jamaicensis* and *B. cavernarum* ate a blend of applesauce, marmoset chow and banana. *Eptesicus fuscus* received a mixture of kitten chow, egg, cottage cheese and banana.

Throughout the experiment, we housed all subjects individually except for *A. jamaicensis*, which responded poorly to housing in isolation. *Artibeus jamaicensis* were housed in pairs for 21.5 h/day and were isolated only for daily 1-h feeding periods and the following 1.5 h. We assigned both members of each pair of *A. jamaicensis* to the same experimental or control condition.

On the 11th day of the experiment, we presented all bats with modified versions of their normal food. *Desmodus rotundus* received bovine blood flavoured with 0.4% weight/volume citric acid, and the other three species received their normal diet to which we added either 0.3% weight/weight cinnamon (*A. jamaicensis* and *B. cavernarum*) or 0.2% weight/weight cinnamon (*E. fuscus*). While foraging in natural habitat, both *A. jamaicensis* and *B. cavernarum* encounter citrus fruits, and taste aversions to these familiar flavours are learned relatively poorly (Revusky & Bedarf 1967; Domjan 1972), making citric acid a poor choice for studies of taste-

aversion learning in these species. Cinnamon (*Cinnamomum* spp.) is derived from the bark of an Old World tree and should, therefore, be unfamiliar to any wild-caught, New World bat.

Each member of all control and experimental groups received intraperitoneal injections equivalent to 2% of their unfed body weight. Members of one control group for *A. jamaicensis*, *B. cavernarum* and *D. rotundus* received injections of physiological saline after they fed on the flavoured food on day 11 (group S, $N=6$; *E. fuscus* not run). Members of the unpaired taste-toxicosis control group (group UC, $N=6$; *D. rotundus* $N=5$) received injections of 1% weight/volume lithium chloride (LiCl) solution immediately after feeding on unflavoured food on day 9 of the experiment.

Members of the two experimental groups received injections of 1% weight/volume LiCl solution after they fed on flavoured food on day 11. Members of one experimental group received injections immediately after the end of the feeding period (immediate-experimental, group I, $N=6$), and members of the other received injections 1 h later (delayed-experimental, group D, $N=6$). Most bats finished feeding well before the end of the 1-h feeding period; so even for group I many minutes had elapsed between ingestion and injection. We monitored all animals for 2 h after injection.

On each of days 12–15, we offered all subjects a choice between flavoured and unflavoured samples of their normal diet for 1 h/day. We measured food intakes daily, corrected for evaporation, and then calculated each subject's mean percentage intake of flavoured diet across the 4 days of testing. We also observed the behaviour of all subjects for 2 h following injection on day 11.

Results and Discussion

On days 12–15, members of all three generalist species in experimental groups (I and D) displayed profound aversions to the cinnamon flavoured diet previously associated with toxicosis, whereas subjects assigned to control groups S and UC continued to ingest appreciable amounts of the cinnamon flavoured diet (Fig. 2). The difference between experimental and control subjects' intake of cinnamon flavoured diet was highly significant (Mann-Whitney U test: *A. jamaicensis*: $U=0$, $N_1=N_2=12$, $P<0.0001$; *B. cavernarum*: $U=4$, $N_1=N_2=12$, $P<0.0001$; *E. fuscus*: $U=2$, $N_1=6$, $N_2=12$, $P<0.001$). In contrast, *D. rotundus* assigned to experimental groups I and D failed to show any toxicosis-induced aversion to citric acid flavoured blood and ingested as much citric acid flavoured blood as did *D. rotundus* assigned to control groups S and UC (Fig. 2; $U=54$, $N_1=11$, $N_2=12$, $P=0.69$).

All 11 *D. rotundus* assigned to control groups S and UC ingested less citric acid flavoured blood than unflavoured blood (sign test: $x=0$, $N=11$, $P<0.001$), indicating, as does the data presented in Fig. 1, that *D. rotundus* easily discriminates citric acid flavoured blood from unadulterated blood. However, during 26 of 44 feeding bouts, the 12 *D. rotundus* in experimental groups consumed greater than 1.0 ml of both adulterated and unadulterated blood. Overall, individual subjects assigned to control and

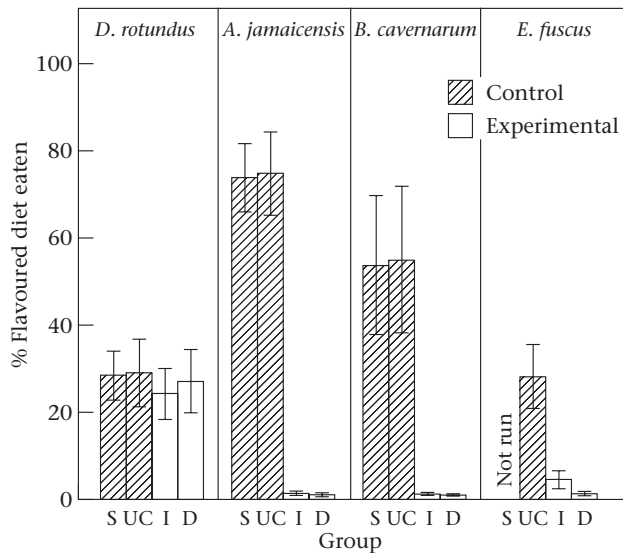


Figure 2. Mean (\pm SE) amount of flavoured diet ingested (expressed as a percentage of the total amount ingested) by subjects in experiment 2 assigned to saline (S) and upaired taste-toxicosis (UC) control groups, and immediate (I) and delayed (D) experimental groups. The left panel describes food choices of common vampire bats and the remaining three panels show food choices of frugivorous and insectivorous bats.

experimental groups ingested, respectively, an average of 16.5 and 16.2 ml of blood each day.

Furthermore, *D. rotundus* subjects were not immune to the emetic effects of lithium chloride. Six of 12 *D. rotundus* assigned to the two experimental conditions vomited following LiCl injection. *Desmodus rotundus*, like the members of the other three species examined, also produced abnormal looking stools and became unresponsive to external stimuli following injection on day 11. Within 45 min of injection with LiCl, vomiting, diarrhoea and other atypical behaviours had ceased in all subjects in all species.

GENERAL DISCUSSION

The results of the present experiment support the hypothesis that generalist bats learn to avoid foods associated with toxicosis. Three species of bat with diverse diets learned taste aversions following a single pairing of a novel flavorant and toxicosis: *A. jamaicensis* and *B. cavernarum*, which are frugivorous bats that should regularly encounter fruits in nature that produce toxic secondary compounds (Cipollini & Levey 1997), and *E. fuscus*, which is an insectivore that would normally be exposed to emetic insects while foraging in natural habitat (Eisner 1970). In fact, like other vertebrate frugivores (Sorenson 1981, 1983; Oates 1987; Handley & Morrison 1991), *A. jamaicensis* appears to follow the phenology of fruit-bearing trees, consuming crops when they become ripe but not while unripe and rich in phenolic compounds and other toxins.

In the laboratory, *E. fuscus* quickly learns to associate white noise with quinine treated mealworms, *Tenebrio*

molitor (Bates & Fenton 1990). Common vampire bats, which are obligate feeders on mammalian blood and therefore are unlikely to experience toxic food in nature, were unable to learn similar associations even under simplified laboratory conditions. This result is striking because sour flavorants are considered especially salient (Kalat & Rozin 1970) and aversions to nonpreferred foods (Figs 1, 2) are typically more pronounced than those to preferred foods (Etscorn 1973; Chambers 1990). We suggest two possible explanations.

Absence of Stabilizing Selection Pressure

Given the evolutionary relationships of the four species used in this study, loss of taste-aversion learning, an otherwise pervasive behavioural character, is the most parsimonious explanation for the lack of taste-aversion learning in common vampire bats. It is possible, although improbable, that secondary neural organizations evolved to 'overwrite' those responsible for taste-aversion learning. A more tenable explanation is that the costs of the neural pathways responsible for taste-toxicosis association in the immediate ancestor of the vampire clade, putatively an insectivorous gleaning bat (Fenton 1992), outweighed the benefits when the switch was made from insects and blood to blood alone. Stabilizing selection would not act to conserve the trait.

Facilitation of Ingestion of Large Quantities of Blood

Alternatively, to evolve as an obligate sanguivore may have necessitated loss of taste-aversion learning. Microchiropteran bats, like humans, have simple stomachs (Neuweiller 2000). For humans, symptoms of blood ingestion (e.g. through injuries to nose or mouth, or bleeding ulcers) include nausea, vomiting and diarrhoea (Braunwald et al. 2002). To facilitate ingestion of large quantities of blood (in extant vampires, 50% or more of body weight per feeding) early vampire bats may have evolved not to form associations between taste and toxicosis. We have no direct evidence supporting this hypothesis. However, *D. rotundus* has the ability to regurgitate facultatively, a behaviour not observed in any nonvampire microchiropteran species. The ability to vomit in the absence of toxicosis would have preadapted early vampire bats for mother-to-pup regurgitation (Wilkinson 1984) and reciprocal altruism (Wilkinson 1984; DeNault & McFarlane 1995).

Conclusion

We have shown, under controlled laboratory conditions, that three bats with generalized diets acquired profound taste-aversions, whereas an obligate blood-feeding bat did not. The comparative design of the experiment suggests that this behavioural trait was lost in the evolution of *D. rotundus*. Our findings are consistent with the hypothesis that taste-aversion learning is an adaptive specialization evolved for poison avoidance. Absence of

this trait in *D. rotundus* allows us to reject the assumption that all mammals, regardless of their diet, retain the ability to learn taste-aversions.

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