



## Ultrasonic vocalizations and social learning of food preferences by female Norway rats

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We examined whether ultrasonic vocalizations facilitate social learning of food preferences in female Norway rats, *Rattus norvegicus*. Females produced ultrasonic vocalizations at higher rates when they were reunited with a familiar conspecific if one member of the reunited pair had eaten an unfamiliar food while the pair members were separated. However, the influence of demonstrator rats on food choices of their observers was as great in pairs of rats that had been devocalized surgically as it was in sham-operated controls. The latter result is not consistent with the hypothesis that ultrasonic vocalizations facilitate social learning of food preferences in Norway rats.

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Members of several rodent species (i.e. Norway rats, *Rattus norvegicus*; house mice, *Mus domesticus*; and Mongolian gerbils, *Meriones unguiculatus*) extract information from conspecifics concerning foods they have recently eaten, and use such information when deciding what foods to eat (e.g. Galef & Wigmore 1983; Valsecchi & Galef 1989; Valsecchi et al. 1996; Galef et al. 1998). Demonstrations of such social acquisition of information are quite straightforward (Galef 2002). Animals are assigned to pairs and a randomly selected member of each pair (the demonstrator) is placed in isolation and fed one of two foods, both of which are unfamiliar to the other member of the pair (the observer). After the demonstrator has eaten, it is placed together with the observer for a few minutes before the observer is offered a choice between the food that the demonstrator ate and a totally unfamiliar food. During the choice test, observers show a significant enhancement of their preferences for whichever of the two foods their respective demonstrators had eaten (see Galef 1988, 1996 for reviews).

The information that observer rats extract from their demonstrators, which influences observers' subsequent food choices, consists of two olfactory components, both carried on the breath of demonstrators (Galef & Stein 1985; Galef et al. 1988). Of course, evidence that olfactory cues on the breath of demonstrator rats (or humans, for

that matter; Galef 2001) alter food preferences of observer rats does not mean that stimuli in other modalities do not facilitate transmission of information from demonstrator rats to their observers.

Olfactory cues necessary for an observer rat to learn what food its demonstrator ate emerge from the nose and mouth of demonstrators, and an observer must approach the head of a demonstrator if it is to detect those cues (Galef & Stein 1985). Consequently, tactile, visual or auditory signals passing between demonstrators and observers might facilitate social learning by increasing the probability that observers could gain access to the relevant olfactory information.

Recently, Moles & D'Amato (2000) showed that the rate of emission of ultrasonic vocalizations (USVs) by mice shortly after they are reunited with familiar companions significantly increases if one pair member eats an unfamiliar food, particularly a palatable one, while separated from its partner. By anaesthetizing one member of each pair of subjects before reuniting them, Moles & D'Amato (2000) showed that animals that were introduced into another's cage produced USVs. And in Moles & D'Amato's experiments, the observer member of a pair was always introduced into the cage where its demonstrator was being held. In discussing these results, Moles & D'Amato (2000, page 693) speculated that 'ultrasonic vocalizations can facilitate proximity, helping relevant information [concerning foods that others have eaten] to be acquired.' They suggested that the 'possible role of USVs in social acquisition of food preferences should be investigated.'

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The hypothesis that USVs may facilitate social learning about foods is consistent with results of experiments showing that USVs, although not necessary for elicitation of sexual behaviour in female rats, appear to facilitate mating (McIntosh et al. 1978; Barfield et al. 1979). However, some studies of USVs produced by adult Norway rats consider them mechanical by-products resulting from thoracic compression caused by forepaw impact during periods of vigorous locomotion (Blumberg 1992). Yet others treat the 50-kHz USVs produced by adult rats to be an index of anticipatory affective states elicited by incentive stimuli (Knutson et al. 2002). On such views, rat USVs are not evolved for and may not serve communicative functions.

In the present study, we show first that pairs of female Norway rats, like those of female house mice, increase their rates of USV following a period of separation during which one pair member had eaten an unfamiliar food. We then examined the role of USVs in social influence on food preference by comparing the magnitude of social influences on food preference in surgically devocalized and sham-operated pairs of rats.

### EXPERIMENT 1: EFFECTS OF UNFAMILIAR FOODS ON RATES OF USV

We undertook experiment 1 to determine whether, in female Norway rats, as in female house mice (Moles & D'Amato 2000), reunited members of separated pairs would show increased rates of USV when one member of a pair ate an unfamiliar food while pair members were apart.

Adult rats produce both long (300–3000 ms) USVs at a frequency of approximately 22 kHz and short (20–80 ms) USVs at a frequency of approximately 50 kHz (Knutson et al. 2002). Low-frequency USVs tend to occur in anticipation of punishment or avoidance, whereas high-frequency USVs are usually produced in anticipation of reward or approach (Knutson et al. 2002). The short 50-kHz USVs of adult rats (like those of mice in the range of 65–75 kHz; Maggio & Whitney 1985) are produced during periods of social investigation, particularly when familiar individuals come into contact (Blanchard et al. 1993; Brudzynski & Pniak 2002).

Taken together, such findings suggest that, if USVs facilitate social learning about foods by rats, the 50-kHz USVs, often produced during amicable social interactions and in anticipation of approach behaviour, are most likely to be involved. Consequently, and because our ultrasonic detector could be tuned to only one frequency at a time, we monitored only 50-kHz USVs of our subjects.

### Methods

#### Subjects

Subjects were 28 7-week-old female Long-Evans rats acquired from Charles River Canada (St Constant, Quebec). Following arrival in the laboratory, subjects remained undisturbed for 1 week to permit recovery from the stress

of transportation and to become familiar with both their pairmate and Teklad Rodent Diet (W) 8640 (diet 8640; Harlan-Teklad, Madison, Wisconsin U.S.A.) to which they had ad libitum access during the week.

Except for the 24 h immediately preceding testing, when subjects were housed individually, they lived in pairs in shoebox cages measuring 20 × 24 × 47 cm, and were maintained in a temperature- and humidity-controlled colony room that was illuminated for 12 h/day (with light onset at 0700 hours).

#### Apparatus

We monitored ultrasound production using a model U30 Bat Detector (Ultra Sound Advice, London, U.K.), Noldus one-channel audio filter (Noldus Information Technology, Leesburg, Virginia, U.S.A.), Noldus UltraVox 2.0 software (Noldus Technologies) and a Compaq Armada 4110 laptop computer.

During testing, a ring stand and clamp held the bat detector over the midpoint of a 10-gallon (37.85-litre) aquarium, measuring 25.4 × 50.8 × 31.8 cm, and the bat detector was set at 55 mHz, with the audio filter at level 7. A lid of half-inch (1.27-cm) hardware cloth prevented subjects from escaping from the aquarium and, to prevent the fingernails of the rats from contacting glass and producing ultrasounds, we lined the floor of the aquarium with disposable, absorbent paper pads (Tray Liners, Lilo products, Hamilton, Ontario, Canada).

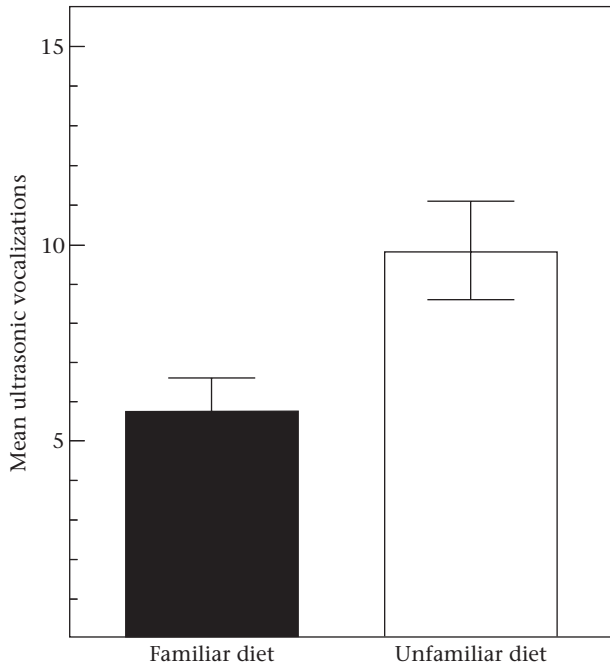
#### Procedure

Twenty-four hours before we measured rate of production of USVs by a pair of subjects, we placed them in separate cages identical to those in which they had been housed together and randomly assigned one member of each pair to the control or experimental treatment. Pair members assigned to the control condition continued to have ad libitum access to diet 8640, while one member of each pair assigned to the experimental condition had access to a bowl containing an unfamiliar casein- and cornstarch-based diet (Normal Protein Test Diet; diet NPT; Harlan-Teklad catalogue number 170590) during the 24-h period of separation.

We reunited a pair of subjects in the aquarium for 3 min and used the Noldus hardware and software to count the number of short 50-kHz USVs each pair of subjects produced while they were together in the test apparatus.

### Results and Discussion

Pair members assigned to the experimental condition (in which one member had eaten diet NPT while pair members were apart) produced significantly more USVs during the 3 min that the reunited pair spent in the test enclosure than did pairs assigned to the control condition (Student's *t* test:  $t_{12} = 2.79$ ,  $P < 0.02$ ; Fig. 1). We conclude that reunited pairs of female Norway rats, like reunited pairs of female house mice, increase their rates of USV production when one member of a separated pair eats an unfamiliar food while pair members are apart.



**Figure 1.** Mean number ( $\pm$ SE) of short (20–80 ms) 50-kHz ultrasonic vocalizations produced by pairs of reunited Norway rats when one member of a pair had eaten an unfamiliar food or both pair members had eaten the same familiar food while pair members were separated.

## EXPERIMENT 2: EFFECTS OF DEVOCALIZATION ON SOCIAL LEARNING

The finding that reunited pairs of Norway rats increased their rates of production of USVs when one pair member had eaten a food unfamiliar to the other suggests that USVs may facilitate exchange of the olfactory information crucial to social induction of food preference (Moles & D'Amato 2000). In the present experiment, we compared the magnitude of social influence on food preference in intact and surgically devocalized pairs of female Norway rats. If USVs play an important role in facilitating social learning about foods, observers that are members of devocalized pairs of rats should show less social influence on their food choices than observers that are members of sham-operated pairs of rats, in which both members can still produce USVs.

### Methods

#### Subjects

Subjects were 28 7-week-old female Long-Evans rats obtained from Charles River Canada. On the last day of testing, subjects were 11–12 weeks old.

#### Apparatus

We used the same apparatus that we had used in experiment 1, as well as stainless-steel hanging cages measuring 21 × 24 × 27 cm and semicircular stainless-steel food cups (10 cm diameter, 5 cm deep).

#### Diets

We composed six different diets (cin, coc, ani, mar, clo, ros) by adding, respectively, 1.0 g McCormick's Ground Cinnamon (McCormick Canada, London, Ontario), 2.0 g Hershey's Pure Cocoa (Hershey Canada Inc., Mississauga, Ontario), 1.0 g bulk ground anise (Horn of Plenty, Dundas, Ontario), Club House Brand Marjoram (McCormick Canada), bulk ground cloves (Horn of Plenty), or bulk ground rosemary (Horn of Plenty) to a sufficient quantity of Teklad Diet 8640 to reach 100 g.

#### Procedure

One to 2 weeks after a subject arrived in the laboratory, we anaesthetized her (intraperitoneal injection with sodium pentobarbital), shaved the ventral surface of her neck, opened an approximately 3-cm incision in the shaved area, and parted the sternohyoidius muscle to expose the trachea. We then either closed the incision (members of seven pairs assigned to the sham-operated condition) or isolated the left inferior laryngeal nerve and removed a 2-mm section of it before closing the incision (members of seven pairs assigned to the experimental condition). Unilateral sectioning of the inferior laryngeal nerve results in cessation of USVs (Roberts 1975; Wetzel et al. 1980; Thomas et al. 1981). For 5 days following surgery, members of each pair resided in individual cages to recover from anaesthesia and surgical insult.

#### Testing for effects of surgery

On the day before we first placed observer rats in the home cages of their demonstrators, to examine effects of devocalization on social learning about foods (see below), we tested all subjects for their production of USVs. To conduct such a test, we placed a pair of demonstrators or a pair of observers, both assigned to either experimental or sham-operated conditions, in the apparatus used in experiment 1, and counted the number of short (20–30 ms) 50-kHz vocalizations produced during the 3 min they remained there.

#### Testing for social learning of food preferences

We used our standard technique (Galef & Wigmore 1983; Galef 2001) to assess effects of ultrasonic devocalization on social learning of food preferences. After we placed members of all 14 pairs of subjects in individual stainless-steel hanging cages, we randomly assigned one member of each pair to serve as a demonstrator, and placed that animal on a restricted feeding schedule, eating diet 8640 for 1 h/day for 2 consecutive days. During these 2 days, each observer continued to eat diet 8640 ad libitum.

After a third 23-h period of food deprivation, for 1 h, we gave diet cin to three demonstrators assigned to the sham-operated condition and three demonstrators assigned to the experimental condition and we gave diet coc to the remaining four demonstrators assigned to each condition.

Immediately after feeding demonstrators, we placed each observer in the cage of its demonstrator pairmate, and allowed demonstrators and observers to interact for 5 min. At the end of the 5-min period of interaction, we

returned observers to their respective home cages and offered them a choice between weighed samples of diets cin and coc for 24 h. At the end of the 24-h choice test, we removed the food cups from each observer's cage, weighed them and determined the percentage of each observer's total 24-h intake that was the diet that its demonstrator had eaten.

In our standard procedure (Galef 2002), we would have terminated the experiment at this point. However, to increase the amount of information acquired from surgically manipulated subjects, we tested social learning in each pair twice more.

After the first test of social learning had been completed, we fed diet 8640 to the observer member of each pair for 24 h and food deprived its demonstrator for 23 h, then fed demonstrators diet 8640 for 1 h. Twenty-three hours later, for 1 h, we fed diet ani to three demonstrators in pairs assigned to both sham-operated and experimental conditions and diet mar to four demonstrators in pairs assigned to sham-operated and experimental conditions. We then allowed each observer to interact with its demonstrator for 5 min, returned observers to their home cages, and offered them a choice between diets ani and mar for 24 h.

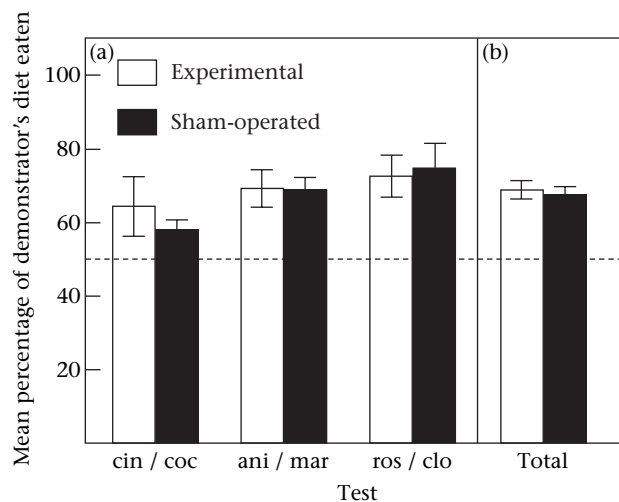
We then repeated the entire procedure once again. This time, after a 1-day respite, we fed diet ros to three demonstrators in each condition and diet clo to four demonstrators before allowing them to interact with their respective observers for 5 min. Following interaction, we returned each observer to its home cage and gave it a choice between weighed samples of diets ros and clo for 24 h.

## Results and Discussion

During the 3-min test preceding social induction of diet preference, all pairs of devocalized rats failed to produce any USVs, whereas sham-operated pairs produced a mean  $\pm$  SE of  $6.8 \pm 0.9$  vocalizations (range 5–9).

There was no difference in the total amount of food eaten during testing by subjects assigned to experimental and sham-operated conditions in any of the three tests (Student's *t* test: all three  $t_{12} < 0.67$ , all NS) or when all three tests were considered together (repeated-measures ANOVA:  $F_{1,12} = 0.20$ , NS).

Based on the hypothesis that USVs facilitate observers' social acquisition of food preferences, devocalization would be expected to reduce the probability that an observer would acquire preferences for foods that its demonstrator had eaten. However, in all three tests in the present experiment, observers assigned to both experimental and sham-operated conditions showed a preference for their respective demonstrator's diet (Student's *t* tests: all  $t_6 > 7.88$ , all  $P < 0.001$ ; Fig. 2). Furthermore, whether we considered the results of each of the three tests of social learning individually (all  $t_{12} < 0.74$ , all NS; Fig. 2) or in combination (repeated-measures ANOVA:  $F_{2,24} = 0.30$ , NS; Fig. 2), there was no sign that sham-operated subjects, which were able to produce USVs, were better able than devocalized experimental subjects to acquire information about foods from their respective demonstrators.



**Figure 2.** Mean percentage ( $\pm$  SE) of observers' 24-h intake that was the same food that their respective demonstrators had eaten when offered a choice between a pair of diets. (a) The results of each test separately. (b) The results of all three tests considered together. Dashed line = chance. Cin = cinnamon-flavoured diet; coc = cocoa-flavoured diet; ani = anise-flavoured diet; mar = marjoram-flavoured diet; ros = rosemary-flavoured diet; clo = clove-flavoured diet.

The apparent increase over trials in the effectiveness of demonstrators in influencing their observers' food choice was not quite statistically significant (linear polynomial test of order:  $F_{1,12} = 3.37$ ,  $P < 0.10$ ) and, if real, probably reflects increasing familiarity with the experimental procedures across trials.

## GENERAL DISCUSSION

The results of the present experiments indicate that ultrasound production in Norway rats, as in house mice (Moles & D'Amato 2000), is increased in reunited pairs if one pair member eats a novel food while away from its partner. However, our data offer no support for the suggestion that USVs facilitate social learning of food preferences (Moles & D'Amato 2000). Of course, the absence of evidence of an effect on social learning found here cannot be taken as evidence of an absence of such effects in general. Possibly, in circumstances other than those examined here, USVs might facilitate observers' exploration of the nose and mouth of their demonstrators and thus facilitate social learning about foods (Galef & Stein 1985). If, for example, demonstrators and observers in the present experiment had been aggressive, adult male rats, rather than relatively amicable young adult females, USVs might have facilitated social learning (Valsecchi et al. 1996). Still, the present results suggest that USVs do not facilitate social learning about foods by young rats, which are believed to be more likely than adults to use information acquired from conspecifics in deciding what to eat (Boyd & Richerson 1985).

If, as the present data suggest, emission of USVs does not facilitate observer rats' social learning about foods from their demonstrators, an interesting question remains as to why observers increase their rate of USV production



when reunited with demonstrators fed an unfamiliar diet. It might be argued that once an observer discovers that its demonstrator has eaten an unfamiliar diet and increases its rate of USV production, it has already been exposed to the olfactory stimuli that alter its subsequent preference for whatever unfamiliar food its demonstrator has eaten. Indeed, results of prior experiments suggest that even very brief periods of nose-to-nose contact between a demonstrator and an observer rat suffice to increase observers' preferences for their respective demonstrators' diets (Galef & Stein 1985).

If the same stimuli that permit observers to identify their demonstrators' diets as unfamiliar are sufficient to alter observers' preferences for that diet, then USVs produced by observer rats after interacting with demonstrators fed an unfamiliar food would be a consequence rather than a cause of social learning about food. If so, USVs produced by observer rats might reflect anticipation of reward, as Knutson et al. (2002) proposed, although that leaves unanswered the question of why observer rats might signal changes in their emotional state following exposure to the smell of an unfamiliar food during interaction with a conspecific demonstrator. It seems unlikely that interaction with a familiar partner that has eaten an unfamiliar food would increase vigour of locomotion sufficiently to produce additional epiphenomenal USVs, as Blumberg (1992) has suggested. Exploration of that hypothesis is beyond the capacities of our laboratory.

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