

## Avoidance of potentially harmful food cannot be socially transmitted between rats

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**Abstract:** The social transmission of food preferences (STFP) is a behavioural task of olfactory memory, in which an observer rat learns safe food odours from a demonstrator rat, and shows preference for this odour in a subsequent choice test. However, previous studies have failed to detect the transmission of information about food of potential danger and food aversion using STFP test. In this study, we tested how demonstrators' health affects the exchange of odour information and whether observers can learn danger information from an unhealthy demonstrator. As expected, the observer rat formed an odour preference after interacting with a demonstrator rat that had just eaten food containing a new odour, however, odour preference rather than aversion was also formed after interacting with a demonstrator rat injected with LiCl (used to induce gastric malaise). Furthermore, anaesthetized demonstrator rats and half-anaesthetized demonstrator rats, which showed obvious motor deficits suggesting an unhealthy state, also socially transmitted food preferences to observers. These results suggest that the social transmission of food preferences task is independent of a demonstrators' health, and that information about dangerous foods cannot be transmitted using this behavioural task.

**Keywords:** Diet selection; Rats; Social learning; Social transmission of food preference; Food aversion

Diet selection is vital for animal survival, especially when they are faced with novel foods (Birch, 1999). Information regarding food safety can be transmitted between individual rats and used for diet selection, as evidenced by behavioural tests such as the social transmission of food preference (STFP) test (Birch, 1999; Galef, 1982, 2003; Posadas-Andrews & Roper, 1983). In the STFP test, an observer rat interacts with a demonstrator rat that has recently eaten food with a novel odour, and learns this odour from the demonstrator rat's breath. When the observer rat is subsequently presented with a choice between the food containing the learned odour or a novel odour it prefers the former (Galef & Whiskin, 2000). This phenomenon suggests a mechanism by which a rat learns safety information about novel food from conspecifics and uses this information when making dietary decisions.

Information about unsafe foods can also be theoretically transmitted via the STFP test. For example, a

demonstrator rat in an unhealthy state may indicate the risk of food recently consumed and an observer may learn food aversion rather than preference from the unhealthy demonstrator. This pattern has been found in red-winged blackbirds (*Agelaius phoeniceus*) (Mason & Reidinger, 1982; Mason et al, 1984) but remains unproven in laboratory rats. In the STFP task, a demonstrator rat made ill by lithium chloride (LiCl) injection or unconscious following anaesthetic administration still induces a food preference in observers only and not a food aversion (Galef & Dalrymple, 1978; Galef, 1982;

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Galef et al, 1983, 1985).

One explanation for this test failing to communicate a social transmission of food preference (STFA) may be because the rat cannot detect the health condition of its poisoned or anaesthetized littermate. Here, we explore this behavioural paradigm further and in addition to LiCl-treated and anaesthetized demonstrator rats, we test half-anaesthetized demonstrator rats showing clear motor deficits and suggesting a severe health condition in a STFP experimental protocol.

## MATERIALS AND METHODS

### Animals

Male Sprague-Dawley rats ( $n=126$ ) weighing 250–300 g were obtained from the animal housing center, Kunming Medical University, Kunming, Yunnan, China. Animals were group-housed in ventilated cages with free access to water and food in a 12-h light/dark cycle and a temperature-regulated environment at the Kunming Institute of Zoology (Chinese Academy of Sciences, Kunming, Yunnan, China). All experimental protocols were approved by the animal ethics committee of the Kunming Institute of Zoology.

### Drugs

LiCl (Sangon Biotech, Shanghai, China) or pentobarbital sodium (Sigma-Aldrich, St. Louis, Missouri, USA) was dissolved in saline. Powdered dill or cinnamon (Fuchs Foodstuffs, Anqiu, Shandong, China) were adulterated to plain chow 1% by weight.

### Social transmission of food preference (STFP) task

The STFP task was performed as previously described (Galef & Whiskin, 2003). Before the task, demonstrator

rats were habituated to eating plain or cinnamon chow for three days, after which demonstrator rats and observer rats were food-deprived for 24 h. During the task, rats underwent a classic three-step procedure. In the first step, the demonstrator rat was allowed 30 min access to plain or cinnamon chow. In the second step, the demonstrator rat was introduced to the observer rat to interact freely for 15 min, during which sniffing was observed. In the third step (15 min after second step) the observer rat was presented with a choice of two foods in its home cage: dill chow and cinnamon chow, both pre-weighed. After 20 min, remaining food was collected and weighed and the amount of each food eaten was calculated as food consumption.

### Data analysis and Statistics

All values are reported as mean $\pm$ SE. A two-tailed unpaired Student's *t*-test or a one-way ANOVA followed by post hoc analysis with least significant difference (LSD) was used for comparisons. The significance level was set at  $P<0.05$ .

## RESULTS

### Formation of a food preference via social learning

We examined the basal food consumption level, wherein after 24 h of food deprivation, rats were presented with plain chow for 20 min (Figure 1A, plain chow,  $n=9$ ,  $4.93\pm 0.34$  g), or presented with dill chow and cinnamon chow to test innate odour preferences (Figure 1A, dill,  $n=9$ ,  $0.82\pm 0.33$  g, cinnamon,  $n=9$ ,  $0.58\pm 0.24$  g). Rats exhibited an innate avoidance to food with a novel odour, consumed much less novel food than familiar food (Figure 1A, plain vs. dill,  $P<0.001$ , plain vs. Cinnamon,  $P<0.001$ ), and showed no difference between dill

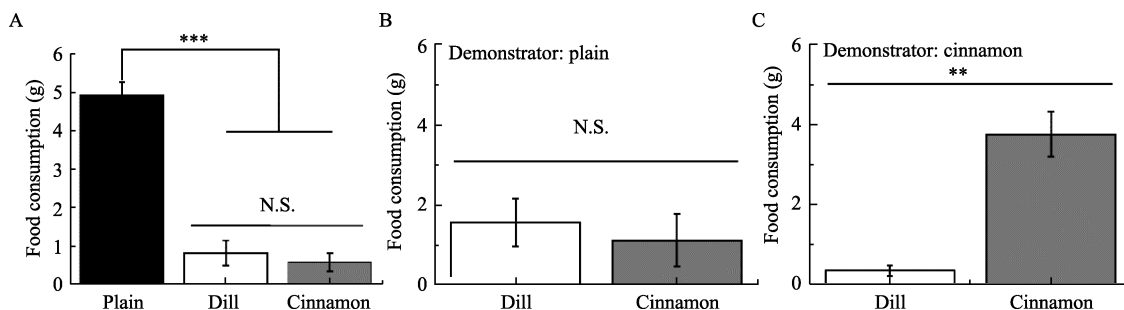


Figure 1 Social transmission of food preference in rats

A: Food consumption level (plain, black bar) and food preference between dill and cinnamon (dill, cinnamon blank and grey bars) after 24 h of food deprivation. B, C: The food preference of observer rats after interaction with demonstrator rats fed plain chow (B) or cinnamon chow (C); N.S.: not significant; \*\*:  $P<0.01$ ; \*\*\*:  $P<0.001$ .

and cinnamon ( $P=0.608$ ). In subsequent experiments, we used cinnamon chow as the odour presented by the demonstrator rats.

We tested the formation of learned odour preferences using a STFP paradigm. After interaction with a demonstrator rat fed with plain chow, the observer rat exhibited food avoidance towards novel food and showed no preference between dill chow and cinnamon chow (Figure 1B,  $n=9$ , dill,  $1.56\pm 0.6$  g, cinnamon,  $1.11\pm 0.65$  g, dill vs. cinnamon,  $P=0.57$ ). Meanwhile, after interaction with a demonstrator rat fed with cinnamon, the observer rat showed a preference towards cinnamon chow (Figure 1C,  $n=9$ , dill,  $0.35\pm 0.13$  g, cinnamon,  $3.76\pm 0.57$  g, dill vs. cinnamon,  $P=0.001$ ). These results suggest that food odour can be socially transmitted and used to guide later food choices.

### Observer rats learn a food preference after interaction with a demonstrator rat poisoned by LiCl

In theory, the odour presented by an unhealthy demonstrator rat may indicate an odour associated with

potentially dangerous food, which is essential for diet selection. Therefore, we tested whether the health condition of the demonstrator rat influences STFP results.

LiCl can induce gut malaise, and intraperitoneal (i.p.) injection of LiCl can serve as an unconditional stimulus in the conditioned taste aversion (CTA) learning paradigm (Bures et al, 1998; Nachman & Ashe, 1973). After feeding on plain chow or cinnamon chow, the demonstrator rat was injected with LiCl solution (127 mg/kg) (Thiele et al, 1997) and allowed to interact with an observer rat before the food preferences of the observer rat was tested. No preference was detected (Figure 2A,  $n=9$ , dill,  $0.9\pm 0.3$ g, cinnamon,  $0.48\pm 0.18$  g, dill vs. cinnamon,  $P=0.20$ ). However, after interaction with a LiCl-injected demonstrator rat fed with cinnamon chow, a preference to cinnamon rather than aversion was detected (Figure 2B,  $n=9$ , dill,  $1.36\pm 0.49$  g, cinnamon,  $4.34\pm 0.58$  g, dill vs. cinnamon,  $P=0.013$ ). Consistent with previous findings, the LiCl-poisoned demonstrator rat and healthy animals transmitted food preferences identically.

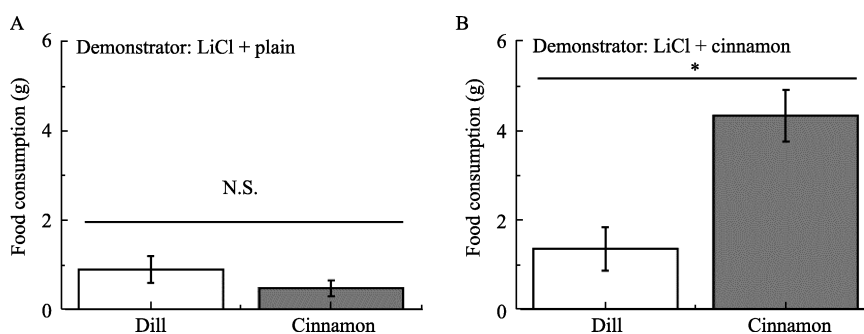


Figure 2 Socially learned food preferences from LiCl-poisoned demonstrator rats

A: After interaction with a LiCl-poisoned demonstrator rat fed plain chow, the observer rat showed no preference between dill and cinnamon chow; B: After interaction with a LiCl-poisoned demonstrator rat fed cinnamon chow, observer rats showed a food preference for cinnamon chow; N.S.: not significant; \*:  $P<0.05$ .

### Food preferences, and not aversions, were socially learned from anaesthetized demonstrator rats

It is possible that the observer rat was unaware of the discomfort felt by the LiCl-injected demonstrator rat, so we used anaesthetized demonstrator rats to induce motor deficits that may more clearly indicate an unhealthy state and food danger.

We anaesthetized the demonstrator rat by injecting pentobarbital sodium (80 mg/kg, i.p.) before interactions. During the interaction session, the demonstrator rat remained inactive and was only able to be sniffed by the observer rat. After interacting with the anaesthetized demonstrator rat fed plain chow, no food preference in

the observer rat was detected (Figure 3A,  $n=9$ , dill,  $0.65\pm 0.19$  g, cinnamon,  $0.65\pm 0.31$  g, dill vs. cinnamon,  $P=0.993$ ), and after interaction with an anaesthetized demonstrator rat fed cinnamon chow a preference for cinnamon was formed (Figure 3B,  $n=9$ , dill,  $0.83\pm 0.33$  g, cinnamon,  $3.53\pm 0.53$  g, dill vs. cinnamon,  $P=0.003$ ).

Next, we half-anaesthetized demonstrator rats by injecting pentobarbital sodium (45 mg/kg, i.p.) before interactions. During the interaction session the half-anaesthetized demonstrator rat showed motor control deficits including staggering, which may also indicate an unhealthy state. We found that after interaction with a half-anaesthetized demonstrator rat fed cinnamon chow

but not plain chow, a food preference for cinnamon was formed by the observer rat (plain chow, Figure 3C,  $n=9$ , dill,  $0.42\pm0.12$  g, cinnamon,  $0.29\pm0.12$  g, dill vs. Cinna-

mon,  $P=0.510$ ; cinnamon chow, Figure 3D,  $n=9$ , dill,  $0.33\pm0.14$  g, cinnamon,  $4.17\pm0.83$  g, dill vs. cinnamon,  $P=0.002$ ).

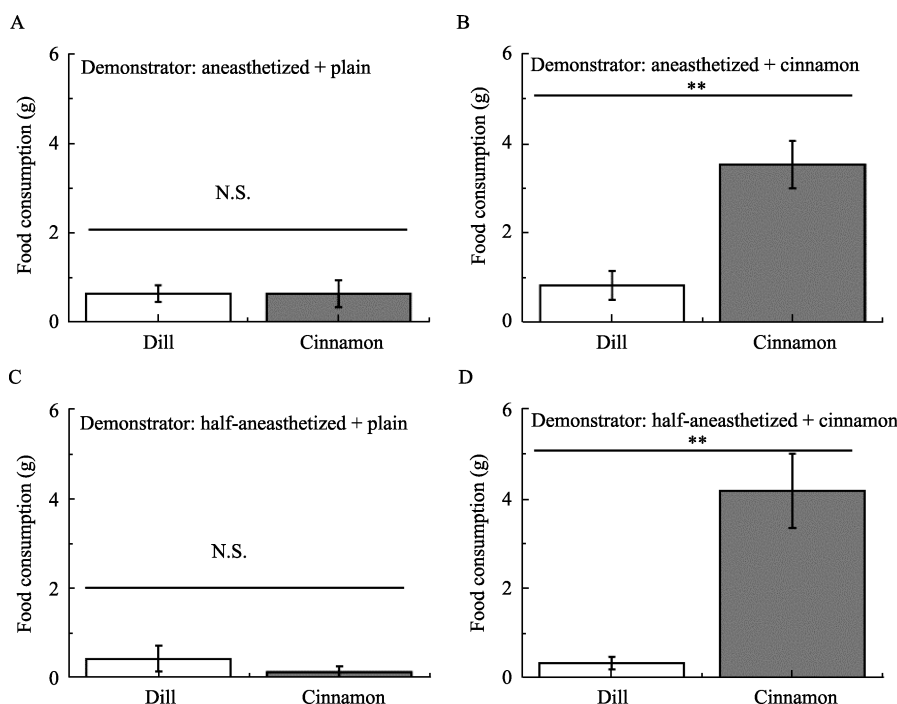


Figure 3 Food preferences learned from anaesthetized or half-anaesthetized demonstrator rats

After interaction with an anaesthetized (A) or half-anaesthetized (C) demonstrator rat fed with plain chow, the observer rat showed no preference for dill or cinnamon chow. After interaction with an anaesthetized (B) or half-anaesthetized (D) demonstrator rat fed cinnamon chow, observers showed a food preference for cinnamon chow. N.S. not significant,  $**P < 0.01$ .

**Total food consumption is an indicator of a STFP and confirms that STFP is independent of the health of the demonstrator**

We compared the total food consumption (dill chow+cinnamon chow) of observer rats in each experiment, and compared this to basal food consumption levels (Figure 4, plain,  $4.93\pm0.34$  g; also see Figure 1A). When rats were first confronted with unfamiliar food, they showed an innate avoidance to that food and ate less than the basal food consumption level (Figure 4, novel,  $1.4\pm0.42$  g, plain vs. novel,  $P<0.001$ ;). We also found that when the observer rat had no food preference (following interaction with a demonstrator rat fed plain chow), total food consumption was significantly lower than the basal level, indicating an unchanged innate avoidance to food with a novel odour (Figure 4, plain demonstrator shown in blank bars, healthy,  $2.68\pm0.6$  g,  $P=0.003$ ; LiCl,  $1.38\pm0.39$  g,  $P<0.001$ ; anaesthetized,  $1.29\pm0.45$  g,  $P<0.001$ ; half-anaesthetized,  $0.71\pm0.16$  g,  $P<0.001$ ; all compared with plain chow). In contrast, when a food preference exists, regardless of the demonstrator rat's

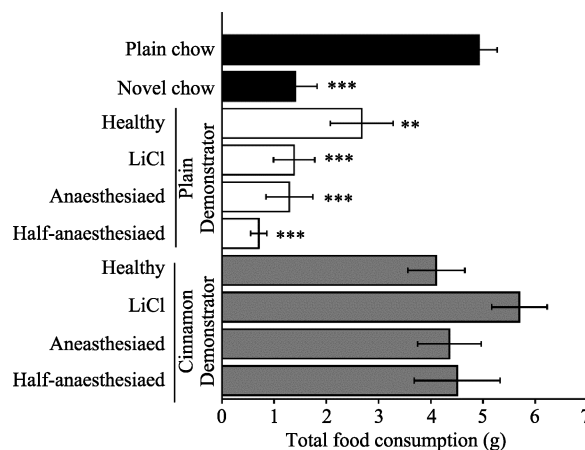


Figure 4 Total food consumption is an indicator of social transmission of food preferences

Total food consumption (dill chow + cinnamon chow) was compared with basal food consumption (plain chow). When rats were presented with novel food (novel chow) they ate less than the basal level. In rats that had interacted with a demonstrator fed plain chow, a food preference was not detected, and total food consumption was less than the basal level. In rats that had interacted with a demonstrator fed cinnamon chow, a food preference was socially transmitted, and total food consumption reached the basal level.  $*:P<0.05$ ;  $**P<0.01$ ;  $***P<0.001$ .

health condition, total food consumption reached the basal food consumption level (Figure 4, cinnamon demonstrator shown in grey bars, healthy,  $4.11 \pm 0.55$  g,  $P=0.26$ ; LiCl,  $5.7 \pm 0.53$  g,  $P=0.29$ ; anaesthetized,  $4.36 \pm 0.61$  g,  $P=0.43$ ; half-anaesthetized,  $4.51 \pm 0.82$  g,  $P=0.56$ ; all compared with plain chow). Thus, total food consumption can serve as an indicator of food preference formation in the STFP task, and using this indicator we found that socially transmitted food preferences are independent of the health condition of the demonstrator.

## DISCUSSION

We demonstrated that a half-anesthetized rat showing obvious motor deficits (suggesting a severe health condition) cannot transmit information about potentially harmful food. On the contrary, observer rats formed a food preference rather than aversion after interaction with these 'unhealthy' rats. We also found that total food consumption can be used as an indicator of formation of STFP. Using this indicator, we confirmed that a socially learned food preference is independent of the demonstrator's health, and food aversion is not transmitted under this paradigm.

One possible explanation is that the observer rat is unable to detect the unhealthy state of the demonstrator rat, the discomfort caused by LiCl, or anaesthetic induced movement deficit or immobility. However, studies indicate that rodents can perceive the discomfort felt by conspecifics, such as in trapped situations, abdominal writhing, formalin induced pain and food shock (Ben-Ami Bartal et al, 2011; Jeon et al, 2010; Langford et al, 2006). Rats also respond differently to

healthy littermates and poisoned littermates (Coombes et al, 1980). Therefore, rather than being unaware of the healthy state of the littermate, rats appear to lack the capacity to use this information to detect potential danger in novel food. Carbon disulphide in rat breathe can induce STFP, and pairing of carbon disulphide with novel odour is sufficient for rats to develop a food preference (Galef et al, 1988). Thus, successful transmission of food preference may solely depend on this semiochemical in rat breath.

Another explanation for a lack of learned food aversion in this test is provided by Bennett Galef (Galef, 1985). According to his theory, socially induced diet avoidance in rats tends to be learned indirectly rather than directly. Rats have an innate tendency to avoid eating food with novel odour (Figure 1A, Figure 4) (Rozin, 1976), and show conditioned taste aversion when experiencing gastrointestinal discomfort after eating novel food (Bures et al, 1998). Therefore, there is little chance for a rat in the natural environment to demonstrate odour following a harmful diet and the odours that can be socially learned by an individual rat are mostly related to safe food (Galef & Clark, 1976). Furthermore, rats can deposit residual olfactory cues in areas they feed and this cue can serve as a safety marker to guide other foraging rats (Galef & Heiber, 1976). Therefore, a lack of STFA may not severely impact the survival of an individual rat.

Ultimately, both explanations are far from satisfactory, and a better strategy is for rats to learn danger information directly from unhealthy littermates so as to avoid potentially harmful unfamiliar food. Alongside with previous findings, our results suggest that rats can learn from others which food to eat but not which to avoid.

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