The Receptor Guanylyl Cyclase Type D (GC-D) Ligand Uroguanylin Promotes the Acquisition of Food Preferences in Mice

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Abstract

Rodents rely on olfactory stimuli to communicate information between conspecifics that is critical for health and survival. For example, rodents that detect a food odor simultaneously with the social odor carbon disulfide (CS₂) will acquire a preference for that food. Disruption of the chemosensory transduction cascade in CS₂-sensitive olfactory sensory neurons (OSNs) that express the receptor guanylyl cyclase type D (GC-D; GC-D+ OSNs) will prevent mice from acquiring these preferences. GC-D+ OSNs also respond to the natriuretic peptide uroguanylin, which is excreted into urine and feces. We analyzed if uroguanylin could also act as a social stimulus to promote the acquisition of food preferences. We found that feces of mice that had eaten odored food, but not unodored food, promoted a strong preference for that food in mice exposed to the feces. Olfactory exploration of uroguanylin presented with a food odor similarly produced a preference that was absent when mice were exposed to the food odor alone. Finally, the acquisition of this preference was dependent on GC-D+ OSNs, as mice lacking GC-D (Gucy2d−/− mice) showed no preference for the demonstrated food. Together with our previous findings, these results demonstrate that the diverse activators of GC-D+ OSNs elicit a common behavioral result and suggest that this specialized olfactory subsystem acts as a labeled line for a type of associative olfactory learning.

Key words: natriuretic peptide, olfaction, olfactory subsystem, social learning

Introduction

Social animals benefit from sharing information that promotes fitness and survival. For example, there is extensive evidence that rodents can transmit dietary preferences to neonates and to peer conspecifics via chemosensory cues (Galef 2012). In many cases, this transmission of food preferences requires active social investigation of “demonstrators” that have consumed particular foods by naïve “observers.” Rats and mice will acquire food preferences from the breath of live conspecifics but fail to acquire preferences to food odors presented on the hind quarters of the other animal (Galef 1985). In contrast, “observer” rats are able to form preferences when allowed to smell, but not contact, anesthetized demonstrator rats dusted with odored foods (Galef 1985). Therefore, the social transmission of food preference requires concurrent detection of both social odors (such as those present in the breath of the demonstrator animal) and odors from a particular food source (eaten by the demonstrator) (Galef...

Rodents also exhibit a preference for food sources that are in close proximity to conspecific social odors such as those present in soiled nest materials (Pastro and Banks 2006; Galef 2012). Rats consistently prefer to eat from a food marked by the excretory products of conspecifics than from an unmarked alternative (Galef and Heiber 1976; Laland and Plotkin 1991), whereas urine marking and the presence of fecal deposits around a food site renders these food sites attractive (Laland and Plotkin 1993). Together, this suggests that rodents find such feeding environments to be beneficial for survival. These benefits could include information about the quantity or quality of nearby food even after conspecifics have vacated the area and would thus offer a useful parallel to information transmitted via more direct social interactions.

There is strong experimental support for a role of the olfactory system in the detection of the social cues necessary for the formation of socially transmitted food preferences (Galef 2012). Carbon disulfide (CS$_2$), an odorous component of rodent breath, can promote the acquisition of food preferences in both rats and mice when paired with a food odor (Bean et al. 1988; Galef et al. 1988; Munger et al. 2010). For example, rats will form food preferences when presented with cotton surrogates to which food odors and 1 ppm (13 μM) CS$_2$ has been added, but will exhibit no preference when the surrogate is supplemented with food odor alone (Galef et al. 1988). This concentration of CS$_2$ specifically activates a specialized subpopulation of olfactory sensory neurons (OSNs) in mice that express the receptor guanylyl cyclase type D isoform (GC-D) (Munger et al. 2010). Perturbation of the sensory transduction cascade in GC-D-expressing (GC-D+) OSNs, such as with the deletion of the gene encoding GC-D ($\text{Gucy2d}^{−/−}$), disrupts olfactory responses to CS$_2$ and prevents mice from acquiring socially transmitted food preferences (Munger et al. 2010).

GC-D+ OSNs respond to a small group of chemostimuli including CS$_2$; urine, a rich source of social stimuli for mice; carbon dioxide; and the natriuretic peptides uroguanylin and guanylin (Hu et al. 2007; Leinders-Zufall et al. 2007; Munger et al. 2010). The guanylin peptides are particularly intriguing candidates as social cues. These peptides are excreted in urine and feces and are thus available for sampling by other animals (Forte 2004). Peptide concentration is linked to feeding, as intestinal secretion of the uroguanylin precursor prouroguanylin is upregulated after a meal and uroguanylin levels in urine are similarly increased postprandially (Forte 2004; Valentino et al. 2011). Both guanylin and uroguanylin are highly efficacious stimuli for GC-D+ OSNs (EC$_{50}$ = 60–770 pM) (Leinders-Zufall et al. 2007), and uroguanylin has been shown to activate GC-D enzymatic activity in a heterologous system (Duda and Sharma 2008). Therefore, we hypothesized that uroguanylin could, like CS$_2$, function as a social cue to promote the acquisition of food preferences.

### Materials and methods

#### Animals

All experimental procedures were approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee. Mice were housed in an AAALAC-accredited laboratory facility. Male C57BL/6J (B6) mice were obtained from the Jackson Laboratory. $\text{Gucy2d}^{+/+}$ and $\text{Gucy2d}^{−/−}$ mice, which have been described previously (Leinders-Zufall et al. 2007; Munger et al. 2010), were maintained in our breeding colony. Mice were group housed (3–4 per cage) in standard cages (28 × 17 × 12.5 cm) with filter-top lids. All mice received water and standard rodent chow ad libitum. The room in which the mice resided was environmentally controlled on a 12:12-h-light:dark cycle (06:00–18:00-h lighting) at a temperature of 21 °C and relative humidity of 50–60%.

#### Food preference testing

Food preference assays were modified from those used previously for testing the social transmission of food preference in rats and mice (Galef et al. 1983; Posadas-Andrews and Roper 1983; Valsecchi and Galef 1989; Crawley 2007; Ryan et al. 2008; Munger et al. 2010). In all experiments, subject mice were pair-housed for 4 days in standard cages with the food container placed on the cage floor. Mice were fed a crushed rodent diet (2018SX; Harlan) to habituate them to powdered food. The amount of food was restricted to 2 g/mouse/day to facilitate feeding during the food preference tests. Food preference was quantified by computing the ratio of the demonstrated food consumed versus the total food consumed by the subject mice (preference ratio [PR] = demonstrated food consumed/total food consumed). All data were expressed as mean ± standard error of the mean. Differences were accepted as significant if $P < 0.05$ (see figure legends for statistical tests).

#### Preference testing after feces exposure

A schematic of the experimental design is presented in Figure 1. B6 mice were randomly assigned as demonstrator or observer mice. Demonstrator mice ($n = 16$) were pair-housed for 5 days and supplied either unadulterated powdered chow or powdered chow adulterated with either cocoa powder (2%; Hershey Co.) or cinnamon (1%; McCormick & Co.). Fecal pellets deposited in the demonstrators’ home cages were collected (0.5–1.0 g) just prior to testing of the observer mice. These fecal pellets were placed in a clean Petri dish (3.5 cm) in a new cage. Individual observer mice were then placed in the cages and allowed to explore for 1 h. Each observer mouse was then moved to a clean cage. After 3 h, observer mice were presented with 2 food trays (3.5-cm Petri dishes mounted to a weighted plastic stage): 1 of which contained cocoa-odored food (3.5 g) and the other contained cinnamon-odored food (3.5 g). Subject mice were allowed to feed for 1 h, at which point the food trays were removed and weighed to calculate the amount of each food consumed. Z-tests (where $z = |$mean
observed PR – 0.50 [standard error of the mean] were performed to determine if there was a statistically significant preference for the demonstrated food (a PR of 0.5 indicates no preference). Significance between stimulus conditions was determined by 1-way analysis of variance (ANOVA).

Preference testing after uroguanylin exposure
A schematic of the experimental design is presented in Figure 2. B6 mice were randomly assigned into 1 of 3 groups (n = 8 each). On the test day, each mouse was moved to individual cages and presented with a Petri dish containing a drop of saline (150 μL) with added flavor powder (2% cocoa or 1% cinnamon) with or without uroguanylin (0, 50 nM or 50 μM). After 1-h exposure, mice were moved to clean cages. After 3 h, mice were presented with 2 food trays (3.5-cm Petri dishes mounted to a weighted plastic stage): 1 of which contained cocoa-odored food (3.5 g) and the other contained cinnamon-odored food (3.5 g). Subject mice were allowed to feed for 1 h, at which point the food trays were

Figure 1 Preference testing with feces. (A) C57BL/6J demonstrator (De) mice eat regular chow (grey) or chow with added odor (cocoa or cinnamon; black). (B) Feces are collected from each demonstrator mouse. (C) Observer (Ob) mice explore feces from the demonstrators. (D) Observer mice are then given the choice of 2 foods: 1 odored with cinnamon and 1 odored with cocoa.

Figure 2 Preference testing with uroguanylin. (A) Observer (Ob) mice (either C57BL/6J or Gucy2d+/− and Gucy2d−/−) explore saline containing a food odor (cocoa or cinnamon) and 50 μM or 50 nM uroguanylin (UG; black) or the food odor alone (grey). (B) Observer mice are then given the choice of 2 foods: 1 odored with cinnamon and 1 odored with cocoa.
removed and weighed to calculate the amount of each food consumed. Z-tests were performed to determine if there was a statistically significant preference for the demonstrated food. Significance between stimulus conditions was determined by 1-way ANOVA.

This experimental paradigm was then used to test the contribution of GC-D to uroguanylin-dependent acquisition of food preferences (Figure 2). Gucy2d+/− and Gucy2d−/− mice (n = 16 each) were each assigned either of 2 groups. Previous studies demonstrated that Gucy2d+/− mice are phenotypically identical to wild-type in their physiological responses to olfactory stimuli (including uroguanylin) and in their ability to acquire socially transmitted food preferences (Leinders-Zufall et al. 2007; Munger et al. 2010). On the test day, each mouse was moved to an individual cage and presented with Zufall et al. 2007; Munger et al. 2010). On the test day, each mouse was moved to an individual cage and presented with a Petri dish containing a drop of saline (150 μL) with added flavor powder (2% cocoa or 1% cinnamon) and uroguanylin (50 nM or 50 μM). After 1-h exposure, subject mice were moved to clean cages. After 3 h, subject mice were presented with 2 food trays (3.5-cm Petri dishes mounted to a weighted plastic stage): 1 of which contained cocoa-odored food (3.5 g) and the other contained cinnamon-odored food (3.5 g). Subject mice were allowed to feed for 1 h, at which point the food trays were removed and weighed to calculate the amount of each food consumed. Z-tests were performed to determine if there was a statistically significant preference for the demonstrated food. Significance between stimulus conditions, between genotype, and for the interaction between stimulus conditions and genotype was determined by 2-way ANOVA.

Results

Rodents prefer to feed in locations where conspecifics have deposited urine and feces (Galef and Heiber 1976; Laland and Plotkin 1991, 1993). We asked if mice can acquire food preferences through interactions with feces of conspecifics. Observer mice were introduced to a food odor through exploration of fecal pellets obtained from a demonstrator mouse that had consumed plain chow or odor chow (containing either 2% cocoa or 1% cinnamon [w/w]). Observer mice were then presented with a choice of 2 powdered chows: 1 containing cocoa and 1 containing cinnamon. Observer mice (n = 8) exposed to feces from demonstrators that consumed food without added cocoa or cinnamon showed no preference for either cocoa- or cinnamon-odored food (Figure 3, Table 1; PR, 0.53±0.05; see figure legends for statistics). By contrast, mice (n = 8) exposed to feces from demonstrators that consumed odor food showed a strong preference for the food containing that same odor (Figure 3, Table 1; PR, 0.74±0.04). These data indicate that feces, like breath, contain social cues that can promote the acquisition of food preferences.

The chemostimulus CS2, when paired with a food odor, is sufficient to increase the attractiveness of foods and to promote the acquisition of food preferences in mice (Bean et al. 1988; Munger et al. 2010). We next tested whether uroguanylin could serve a similar role. Individual observer mice were allowed to interact with a Petri dish containing an odor (2% cocoa or 1% cinnamon) with or without uroguanylin (50 nM or 50 μM) in saline. Each mouse was subsequently presented with a choice of 2 odorized chows (cocoa vs. cinnamon). Observer mice exposed to odorized saline without uroguanylin (n = 8) showed no preference for cocoa- or cinnamon-odored food (Figure 4, Table 1; PR, 0.53±0.05). However, mice exposed to saline containing either concentration of uroguanylin (n = 8 each) showed a strong preference for food containing the demonstrated odor (Figure 4, Table 1; 50 nM uroguanylin: PR, 0.77±0.04; 50 μM uroguanylin: PR, 0.82±0.05). Together, these results show that uroguanylin, a component of both feces and urine, can promote the acquisition of food preferences.

Finally, we asked if GC-D+ OSNs mediate uroguanylin-dependent acquisition of food preferences. We would predict this to be the case as GC-D+ OSNs are sensitive detectors of uroguanylin and mediate the acquisition of food preferences in response to interactions with both live demonstrators and CS2 (Leinders-Zufall et al. 2007; Munger et al. 2010). We assessed the ability of Gucy2d+/− mice (which do not produce GC-D) or their heterozygous controls to acquire food preferences after exposure to a food odor (cocoa or cinnamon) in the presence of either 50 nM or 50 μM uroguanylin. Similar to B6 mice, Gucy2d+/− mice...
Uroguanylin Promotes the Acquisition of Food Preferences in Mice

(n = 8 each group) exhibited a strong preference for food containing the demonstrated odor (Figure 5, Table 1; 50 nM uroguanylin: PR, 0.77 ± 0.05; 50 μM uroguanylin: PR, 0.86 ± 0.04). However, Gucy2d−/− mice (n = 8 each group) showed no preference for either the demonstrated or novel odor (Figure 5, Table 1; 50 nM uroguanylin: PR, 0.54 ± 0.04; 50 μM uroguanylin: PR, 0.51 ± 0.03). Thus, GC-D, and the OSNs that express it, are required for the acquisition of uroguanylin-dependent food preferences.

Table 1 Amount of food consumed (g) by observer mice in food preference assays (mean ± standard error of the mean)

<table>
<thead>
<tr>
<th>Stimulus and food consumed</th>
<th>Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C57BL/6J</td>
</tr>
<tr>
<td>Feces (with odor)</td>
<td></td>
</tr>
<tr>
<td>Total food</td>
<td>2.22 ± 0.25</td>
</tr>
<tr>
<td>With demonstrated odor</td>
<td>1.64 ± 0.21</td>
</tr>
<tr>
<td>With novel odor</td>
<td>0.58 ± 0.10</td>
</tr>
<tr>
<td>Feces (without odor)</td>
<td>1.30 ± 0.11</td>
</tr>
<tr>
<td>Total food</td>
<td></td>
</tr>
<tr>
<td>With cocoa</td>
<td>0.61 ± 0.09</td>
</tr>
<tr>
<td>With cinnamon</td>
<td>0.68 ± 0.09</td>
</tr>
<tr>
<td>Odor alone</td>
<td>0.94 ± 0.13</td>
</tr>
<tr>
<td>Total food</td>
<td>0.49 ± 0.09</td>
</tr>
<tr>
<td>With demonstrated odor</td>
<td>0.45 ± 0.09</td>
</tr>
<tr>
<td>With novel odor</td>
<td>0.45 ± 0.09</td>
</tr>
<tr>
<td>Odor + UG (50 μM)</td>
<td>1.50 ± 0.26</td>
</tr>
<tr>
<td>Total food</td>
<td>1.15 ± 0.14</td>
</tr>
<tr>
<td>With demonstrated odor</td>
<td>0.35 ± 0.12</td>
</tr>
<tr>
<td>With novel odor</td>
<td>0.22 ± 0.07</td>
</tr>
<tr>
<td>Odor + UG (50nM)</td>
<td>1.42 ± 0.19</td>
</tr>
<tr>
<td>Total food</td>
<td>1.08 ± 0.14</td>
</tr>
<tr>
<td>With demonstrated odor</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td>With novel odor</td>
<td>0.50 ± 0.11</td>
</tr>
</tbody>
</table>

UG, uroguanylin; odor: either 2% cocoa or 1% cinnamon (counterbalanced).

Discussion

The mammalian olfactory system responds to a diverse array of chemical stimuli including gases, volatiles, and even peptides and proteins. Many of these chemicals activate specialized olfactory subsystems and elicit specific behaviors or physiological changes (Munger et al. 2009). We previously reported that uroguanylin activates a subpopulation of sensory cells, GC-D+ OSNs (Leinders-Zufall et al. 2007). Furthermore, we found that GC-D+ OSNs mediate the acquisition of socially transmitted food preferences in response to the social stimulus CS2 (Munger et al. 2010). Here, we extend those findings to show that both feces and the GC-D agonist uroguanylin can promote the acquisition of food preferences.

Both feces and urine are rich sources of conspecific and heterospecific semiochemicals that can carry critical information about social or reproductive status or the presence of predators or competitors (Chamero et al. 2007; Papes et al. 2010; Roberts et al. 2010; Ferrero et al. 2011; Isogai et al. 2011). Furthermore, mice will reduce feeding in areas containing feces and urine of competitors (Dobly et al. 2001). GC-D+ OSNs respond to urine with an increase in intracellular Ca2+ and action potential firing (Leinders-Zufall et al. 2007). Uroguanylin is produced in the intestine, where it can act locally to promote the absorption of electrolytes (Forte 2004; Seeley and Tschöp 2011). Its function in the kidney is similar (Forte 2004; Seeley and Tschöp 2011). Upon excretion in urine or feces, uroguanylin is available to act as a semiochemical for other animals that encounter fecal or urine deposits. Our results showing that feces contains cues that, when paired with a food odor, can promote the acquisition of food preferences is consistent with a role for uroguanylin as a chemosensory cue important for social learning in rodents. The recent observations that...
Socially transmitted food preferences prompt the recipient animal to preferentially ingest food that has been safely eaten by conspecifics (Galef 2012). It would thus seem to be biologically advantageous for rodents to use a number of diverse stimuli to activate cells that mediate such an important behavior. For example, peptide stimuli are somewhat stable and are thus likely to persist in fecal or urine deposits long after the donor animal has left the vicinity. By contrast, volatiles and gases should be more easily dispersed with the breath during conspecific interactions. GC-D+ OSNs are differentially sensitive to stimuli of these different classes. The peptides guanylin and uroguanylin are both effective stimuli at low nanomolar concentrations (Leinders-Zufall et al. 2007). GC-D+ OSNs are also responsive to the volatile CS₂, albeit at high nanomolar concentrations (Munger et al. 2010); at higher concentrations (>1 μM), CS₂ will also stimulate canonical OSNs (Munger et al. 2010). The least effective stimulus for GC-D+ OSNs is CO₂ gas, which only activates these cells at concentrations above ~10 mM (Hu et al. 2007; Munger et al. 2010). The ability of GC-D+ OSNs to function as multimodal chemodetectors (Zufall and Munger 2010) may be particularly useful when there is a need to detect relevant social cues during active investigation of other animals or in socially complex environments that are intermittently occupied by conspecifics, competitors, or predators. Furthermore, the observation that both guanylin and CS₂ stimulate GC-D+ OSNs and promote the acquisition of food preferences suggest that these cells are part of an olfactory “labeled line” dedicated to detecting and encoding chemostimuli that promote this specific type of associative social learning.

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References


Laland KN, Plotkin HC. 1993. Social transmission of food preferences among Norway rats by marking of food sites and by gustatory contact. Anim Learn Behav. 21:35–41.


