Sugar-Conditioned Flavor Preferences in Normal and Sweet “Tasteless” Mice

Abstract
The sweet taste of sugars is thought to be the primary determinate of intake and preference. However, sugars also have post-oral effects in the gut that may affect sugar preference. This study used the conditioned flavor-preference paradigm with sweet “tasteless” and normal mice to study the oral and post-oral actions of three different sugars, glucose, fructose, and galactose. Trpm5 knockout mice (KO), with non-functioning sweet taste cells, and normal mice (B6) were trained 24 hr/day with flavored (CS+) 8% sugar solutions and flavored (CS-) water. The mice were then given two-bottle tests with the CS+ vs. CS- flavors in water only, followed by sugar vs. water tests with no added flavors. The B6 mice preferred all CS+ flavors to CS- flavors and all three sugars to plain water. The B6 mice consumed more glucose than fructose and galactose. The KO mice preferred both glucose- and galactose-paired CS+ flavors to CS- flavors, as well as both sugars to plain water. They did not, however, prefer the fructose-paired CS+ flavor and only weakly preferred plain fructose. The KO mice consumed more glucose than galactose, and more galactose than fructose. The KO data indicate that the relative potency of the sugars’ post-oral effects is glucose > galactose > fructose. Yet, B6 data from this and other studies indicate that the relative sweetness of the sugars is fructose > glucose > galactose. Ultimately, both taste and post-oral effects determine sugar preference.

Introduction
Sugar consumption can be hard to discourage since the rewarding effects of ingesting sweets can be paired with the flavors the sweets are taken with. This aids in increasing preference for those flavors associated with potent sugars. For example, when mice drink a cherry flavored drink paired with a sugar with potent post-oral effects, it is likely that they will develop a preference for the cherry flavor. Previous data has shown that sugars such as fructose, glucose, and galactose, are all capable of shifting flavor preference in both mice and rats. Sugars with great reinforcing effects, can increase the preference for sugar paired flavors, and even other sugars. Those without reinforcing effects may not be capable of inducing a preference sugar-paired flavors.

The goal of this study is to compare the post-oral effects of fructose, glucose, and galactose. The potencies of these sugars are determined by examining the amount of solution ingested. Intake and post-oral effects are positively correlated, so it assumed that the greater the intake, the greater the post-oral effect.

Normal wild type mice cannot be used independently to study post-oral effects. All that can be deduced from normal mice preferring a sugar paired flavor over a water paired flavor is that the taste of the sugar combined with the sugar’s post-oral effect has reinforcing capabilities. Tasteless mice must be used to eliminate the effect of the sugar. When tasteless mice prefer a sugar paired flavor over a water paired flavor, the positive reinforcement is due to the post-oral effects of the sugar and not the taste of the sugar.
A previous study done with rats, paired flavors with intragastric fusions of fructose, glucose, galactose, or water (control). The study displayed that rats did not prefer fructose, but did preferred glucose and avoided galactose. The sugars were infused directly into their stomach in order to bypass the mouth and avoid taste-based preference. This technique however goes against nature. In normal consumption, sugars pass through the throat and esophagus, but in the study, the sugars were infused directly into the stomach. If there were any interactions between the sugar and the digestive tract there that may be active in the reinforcing effects of the sugar, IG infusions would not be able to take them into account. The unnatural infusions themselves may even indirectly affect preference. To avoid these complications we will instead use tasteless KO mice.

*Trpm5* mice are also known as sweet tasteless mice because they are unable to detect the taste of sweets. *Trpm5* ion channel is a downstream regulator in the sweet taste receptor cell, responsible for depolarizing the cell, in response to sweets. *Trpm5* KO mice are unable to taste sweets so we expect them to prefer fructose and glucose and avoid galactose, like IG rats.

By understanding the means by which sugars…, we can soon learn how to inhibit this process, thus lowering. Disease prevention,

**Procedure**

*Training*. On the first day, the mice drank (CS-0.05% Kool-aid). Solutions were made fresh each day. The CS- flavors were randomly assigned. The following day, the mice were given CS+ (0.05% Kool-aid and 8% sugar). This cycle was repeated, making a total of four days of training, two days with CS- and two days with CS+/sugar. To reduce the effects of side preference, the mice had experience with both flavors on both sides.

*Testing*. On testing days the mice were given a choice either the CS+ or CS- flavor alone in water. To reduce the effects of a side preferences, the bottles were placed on different side the following day.

*Measurements*. The INITIAL recorded mass is the mass of the full bottles on a spill tray. A few drops of solution is allowed to drip into the tray, then the bottles are placed in the cage tops. The mass of the TRAY and the few drops of spilled solution are then recorded. The next day, the bottles are taken down and the mass of the depleted bottle is taken (END).

Each experiment had two spill bottles. Spill bottle were placed atop empty cages and are meant to determine how much solution would naturally spill. The intake is calculated by subtracting the tray, the spill, and the end weight, from the initial weight of the bottle.

**Results**
**Fructose.** During one bottle trainings, the tasteless mice drank an average of 5.8g of CS+/sugar and 5.75 CS-/water. In a two bottle test, the tasteless mice showed little preference for the CS+ flavor (62, 59 %) over CS- and some preference for fructose (55, 79%). During one bottle trainings, the normal mice drank an average of 14g of CS+/sugar and 5.2 CS-/water. In a two bottle test, the normal mice showed a preference for the CS+ flavor (82, 66 %) over CS- and a preference for fructose (94, 97%).

**Glucose.** During one bottle trainings, the tasteless mice drank an average of 20g of CS+/sugar and 6g CS-/water. In a two bottle test, the tasteless mice showed preference for the CS+ flavor (97, 87%) over CS- and some preference for glucose (85, 96%). During one bottle trainings, the normal mice drank an average of 31g of CS+/sugar and 4.5 CS-/water. In a two bottle test, the normal mice showed a preference for the CS+ flavor (85, 66 %) over CS- and a preference for glucose (99, 99%).
Galactose. During one bottle trainings, the tasteless mice drank an average of 13.7g of CS+/sugar and 5.75 CS-/water. In a two bottle test, the tasteless mice showed little preference for the CS+ flavor (82, 65 %) over CS- and some preference for galactose (79, 92%) During one bottle trainings, the normal mice drank an average of 16.5g of CS+/sugar and 5.9 CS-/water. In a two bottle test, the normal mice showed a preference for the CS+ flavor (87, 62 %) over CS- and a preference for galactose (90, 94%)

Discussion

The KO mice showed no preference for fructose but the B6 mice did. Fructose must have a sweet taste and a little or no post-oral effects. Previous data show that in 1 minute tests with fructose, (rats or mice) did in fact prefer fructose. In 1 minute tests the mice do not experience any post-oral effects so there choice is based solely on taste. This explains why even though fructose has no post-oral effects, the B6 mice were still able to prefer CS+, the sweet taste of fructose is responsible for its reinforcing capabilities. When the taste of fructose cannot be detected, the KO mice drink almost as much fructose as water and as much CS+/fructose as CS-/water. This is consistent with other studies where neither KO mice nor IG infused rats preferred fructose to water.

Both the KO mice and the B6 developed a preference for glucose. Glucose must have potent post-oral effects. In both the rat and mice model, preference for glucose paired flavors can be acquired without the taste of glucose. Rats receiving IG infusions of glucose paired with a neutral flavor develop a preference for the CS+ flavor. The post-oral effects of glucose are so strong that we believe they are capable of increasing preference for other sugars as well. One study has shown that KO mice that previously had no preference for fructose, develop preference for fructose after glucose experience. Both the KO mice and B6 develop some preference for CS+/galactose flavor. This was unexpected because the rats avoided both CS+ and galactose. Galactose must have post-oral effects.

The relative potencies of the post-oral effects of the sugars fructose, glucose, and galactose according to the data is glucose>galactose>fructose.

It is possible that differences in the mice and rat micro flora are responsible for the difference in galactose consumption. There maybe a variety of microorganisms in the
mice gut that allows them to effectively metabolize galactose. Another possible explanation is that galactose may in fact be sweet to rats, however their may be negative reinforcing effects that are strong enough to make galactose aversive to rats. We used 24hr test in order to observe the post-oral effects. A short term study (1 min test) with food restricted rats may help us determine how sweet galactose actually is to rats. If rats in a 1min study continue to avoid galactose, then we the difference rat and mouse taste receptor could be responsible for why rats avoid and mice prefer galactose. If the rats prefer galactose in a short term test this tells us that galactose is sweet yet has aversive post-oral effects. This could explain why galactose intake seems to decrease with experience. In one CFP study, rats drank significantly less galactose on the second day of training than on the first. Another CFP study measured the galactose intake both pre-training and post training. Galactose intake greatly decreases after galactose experience. Initial contact with galactose, due to its possible sweet taste, may be pleasant to rats until they experience its negative post-oral effects.