Chemical Senses, 2024, **49**, 1–15
https://doi.org/10.1093/chemse/bjae001
Advance access publication 6 January 2024
Original Article

Original Article

TRPM4 and PLCβ3 contribute to normal behavioral responses to an array of sweeteners and carbohydrates but PLCβ3 is not needed for taste-driven licking for glucose

Verenice Ascencio Gutierrez[1,†], Laura E. Martin[2,†], Aracely Simental-Ramos[3], Kimberly F. James[1], Kathryn F. Medler[4], Lindsey A. Schier[3,*], Ann-Marie Torregrossa[1,5,*]

[1]Department of Psychology, State University of New York at Buffalo, Buffalo, NY 14260, United States
[2]Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331, United States
[3]Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089, United States
[4]Department of Cell and Molecular Biology, Virginia Tech, Blacksburg, VA 24061, United States
[5]University at Buffalo Center for Ingestive Behavior Research, Buffalo, NY 14260, United States

[†]These authors contributed equally to this work.
[*]Corresponding authors: Department of Psychology, 204 Park Hall, State University of New York at Buffalo, Buffalo, NY 14260, United States. Email: amtorreg@buffalo.edu; Department of Biology, 3616 Trousdale Pkway, AHF 252, University of Southern California, Los Angeles, CA 90089, United States. Email: lschier@usc.edu

The peripheral taste system is more complex than previously thought. The novel taste-signaling proteins TRPM4 and PLCβ3 appear to function in normal taste responding as part of Type II taste cell signaling or as part of a broadly responsive (BR) taste cell that can respond to some or all classes of tastants. This work begins to disentangle the roles of intracellular components found in Type II taste cells (TRPM5, TRPM4, and IP_3R3) or the BR taste cells (PLCβ3 and TRPM4) in driving behavioral responses to various saccharides and other sweeteners in brief-access taste tests. We found that TRPM4, TRPM5, TRPM4/5, and IP_3R3 knockout (KO) mice show blunted or abolished responding to all stimuli compared with wild-type. IP_3R3 KO mice did, however, lick more for glucose than fructose following extensive experience with the 2 sugars. PLCβ3 KO mice were largely unresponsive to all stimuli except they showed normal concentration-dependent responding to glucose. The results show that key intracellular signaling proteins associated with Type II and BR taste cells are mutually required for taste-driven responses to a wide range of sweet and carbohydrate stimuli, except glucose. This confirms and extends a previous finding demonstrating that Type II and BR cells are both necessary for taste-driven licking to sucrose. Glucose appears to engage unique intracellular taste-signaling mechanisms, which remain to be fully elucidated.

Key words: taste, sweet, glucose, starch, TRPM4, PLCβ3.

Introduction

Taste receptor cells (TRCs) use a variety of receptors and signaling mechanisms to transmit information about the chemical properties of foods and fluids, which are then used to increase or decrease ingestive motivation accordingly. Traditionally, Type II TRCs are thought to be responsible for G-protein-coupled receptor-based transduction of bitter, umami, and sweet (BUS) stimuli. For example, sugars, low calorie sweeteners, and some proteins and amino acids bind to the heterodimer receptor made up of T1R2 and T1R3, which, in turn, activates phospholipase C, isoform β2 (PLCβ2), cleaves phosphatidylinositol 4,5-biphosphate (PIP_2), and produces 1,3,4-inositol triphosphate (IP_3) in sequence. IP_3 then binds to IP_3 receptor Type III (IP_3R3) causing the release of calcium (Ca^{2+}) from internal stores. For many years, it was thought that Ca^{2+} initiated the influx of sodium into Type II cells via a single channel, the transient receptor potential melastatin 5 (TRPM5) channel, but we recently demonstrated that transient receptor potential melastatin 4 (TRPM4) is also found in TRCs, and contributes to sweet taste transduction (Dutta Banik et al. 2018, 2020).

Type III TRCs are traditionally thought to contribute to the sensing of salts and acids. However, in a recent study, we described a subset of what appear to be Type III cells that are required for normal BUS taste responses. These TRCs respond to acid and membrane depolarization with a Ca^{2+} signal that identifies the presence of voltage-gated Ca^{2+} channels in Ca^{2+} imaging preparations, as would be expected for Type III cells. These TRCs express phospholipase C isoform 3 (PLCβ3) as opposed to the PLCβ2 expression in Type II cells (Dutta Banik et al. 2020). While individual Type II cells respond to a single quality of taste stimuli (e.g. bitter, sweet, or umami stimuli), the PLCβ3-expressing cells respond to multiple taste qualities. Therefore, we designated this subset of taste cells as "broadly responsive" (BR) (Dutta Banik et al. 2020). Loss of function of either the Type II cells (through a knockout [KO] of IP_3R3) or the BR cells (through a KO of PLCβ3) resulted in

Editorial Decision 15 December 2023.

mice that treated BUS stimuli like water in brief-access taste tests (Dutta Banik et al. 2020).

To date, the involvement of these 2 novel taste-signaling proteins, TRPM4 and PLCβ3, have only been investigated with a select number of representative stimuli, including a limited array of carbohydrates. Carbohydrates are chemically, metabolically, and, in some cases, perceptually, diverse. Glucose, in particular, is a unique stimulus. Multiple lines of evidence now suggest that rodents and humans may employ multiple mechanisms to detect glucose, including a pathway that is T1R2/3 receptor independent. For instance, glucose can elicit a cephalic-phase insulin release (CPIR) even in mice that lack T1R3 (Glendinning et al. 2015). Furthermore, in 1-min, 1-h, and 24-h 2-bottle preference tests, T1R3 KO mice lick more for glucose over fructose (Sclafani et al. 2020). Although one cannot exclude the possibility that T1R2 alone could contribute to some signaling, these data suggest that the T1R2/3 heterodimer is not necessary for glucose responding. Others have suggested that even though glucose sensing does not require T1R3 in this context, it may still depend on signaling in Type II cells (Glendinning et al. 2017). Additionally, previous work has found that genetically modified mice that lack a functional T1R2/3 receptor or the TRPM5 channel are still able to discriminate between glucose and fructose after having had extensive experience associating oral with postoral effects of each respective sugar (Schier and Spector 2016; Schier et al. 2019; Ascencio Gutierrez et al. 2022). Together, these data indicate that the signaling pathways used by other monosaccharides, disaccharides, and artificial sweeteners may not be the only ones used to detect glucose.

Starch is a general term referring to oligo- and polysaccharides, composed of chains of bonded glucose units. Many of the starches used in food production or sensory testing are actually maltodextrins, or commercially produced starch hydrolysis products of varying chain lengths. Rodents prefer many of these compounds to water, but do not necessarily treat them as having the same perceptual quality as sugars and sweeteners (Sclafani 1987, 1988; Sclafani et al. 1987; Elizalde and Sclafani 1988; Ramirez 1991; Lapis et al. 2014, 2016; Smith and Spector 2017). Recent research has demonstrated that humans can discriminate maltodextrin from tasteless gum, suggesting that texture is not necessarily a discriminant factor (Lapis et al. 2014, 2016). Multiple lines of evidence suggest that larger carbohydrates may be detected in a way that is distinct from mono-, di-, and trisaccharides. Maltodextrin administration elicits different neural response patterns in the rat nucleus of the solitary tract compared with sucrose (Giza et al. 1991) and genetic deletion of T1R3 in mice diminishes motivated behaviors for sucrose, with only minor consequences for maltodextrin (Treesukosol et al. 2009; Zukerman et al. 2009, 2013; Treesukosol and Spector 2012). Furthermore, humans can taste maltooligosaccharides, and this detection is independent of hT1R2/hT1R3 (Pullicin et al. 2017; Andrewson et al. 2023). Interestingly, loss of TRPM5 disrupts maltodextrin preference in mice, suggesting that although the canonical sweet receptor is not necessary for response to oligo- or polysaccharides, it does rely on some of the signaling machinery typically found in Type II cells (Zukerman et al. 2013).

The goal of this work is to investigate the role of these newly described signaling components in sugar and maltodextrin taste in mice. We tested IP_3R3 KO, PLCβ3 KO, TRPM4 KO, TRPM5 KO, TRPM4/5 double (D) KO, and multiple types of wild-type (WT) mice on a range of sugars, sweeteners, and maltodextrins to determine if carbohydrates engage heterogenous signaling mechanisms to instigate their unique motivational features. Please note that references to BUS, or the individual taste qualities themselves {e.g. "bitter," "sweet," "umami," etc.} are flawed when discussing animal work. We can never be sure that the perceptual experience of a rodent is identical to that of the human experience described by these adjectives.

General methods

Subjects

Both sexes were used for all experiments, and mice ranged in age from 2 to 12 mo. The colony room was maintained at 20 ± 2 °C with a 12:12-h light/dark cycle. All training and testing were performed during the lights-on phase. Mice were group housed throughout the experiments in standard polycarbonate cages (7.5″ × 11.5″ × 5″). All mice had ad libitum access to water and rodent chow (Envigo 2018, Inotiv, Indianapolis IN), except when water deprivation is indicated for experimental purposes. Animals were cared for in compliance with the University at Buffalo Institutional Animal Care and Use Committee.

Mouse lines

The PLCβ3 KO mouse was generously provided by Dr. Sang-Kyou Han (Han et al. 2006) and was generated in a 129SV agouti mouse strain that was crossed with CD1 mice (Xie et al. 1999). The IP_3R3 KO mouse model was generated in a C57BL/6 background and was obtained from the Mutant Mouse Resources and Research Center (MMRRC:032884-JAX) (Hegg et al. 2010). TRPM4 KO, TRPM5 KO, and TRPM4/TRPM5 DKO were generated on a C57BL/6 background. TRPM4 KO mice were generated by Dr. Marc Freichel (Dutta Banik et al. 2018). TRPM5 and TRPM4/TRPM5 DKO transgenic mice were generously provided to Dr. Kathryn Medler by Dr. Robert Margolskee of Monell Chemical Senses Center. All mice were bred at the University at Buffalo.

Brief-access licking methods

Apparatus (Davis rig)

To determine whether any of the signaling proteins of interest affected taste responding, we recorded licking responses to varying concentrations of stimuli in the Davis rig (Davis MS80 Rig; DiLog Instruments and Systems, Tallahassee, FL, and Med Associates MED-DAV-160, Fairfax, VT) (Smith 2001). Mice are placed in a Plexiglas chamber with a wire grid floor. At the front of the chamber, there is a small opening where the animal can access one of sixteen spill-proof drinking tubes that reside on a sliding platform. Access to these bottles is controlled via a mechanical shutter in between the cage and bottle. The computer-controlled shutter opens and closes to allow the mouse access to one of the tubes for a user-specified length of time. During testing, the shutter is opened at the beginning of a trial and kept open (allowing access to the presented bottle) until the animal chooses to lick at the bottle. Once the animal starts licking, it is allowed 10 s access to the

bottle, after which the trial ends, the shutter occludes access to the bottle, and the sliding platform moves to a different bottle, as decided by the program. Following this, the next trial begins as the shutter opens. The intertrial interval (ITI) is 10 s for all experiments except when noted otherwise. A computer controls the movement of the platform, order of tube presentation, opening and closing of the shutter, duration of tube access, and the interval between tube presentations. Each individual lick is detected by a contact lickometer and recorded on a computer via DavisPro collection software (DiLog Instruments and Systems and Med Associates).

Brief-access sugar training

Mice were adapted to the test chamber and trained to drink from the sipper tubes for 5 consecutive days as previously described (Torregrossa et al. 2014; Martin et al. 2018). During training, mice were 20-h water deprived. On the first day of training, mice were presented with a single stationary bottle of water for 30 min, and the total number of licks to that bottle was recorded. In order to move forward in the training procedure, mice were required to lick a sufficient number of times (200 licks) at the stationary bottle, indicating that they were able to find the stimuli presented.

On the second day, the Davis rig was outfitted with 8 tubes containing 0.25 M sucrose. A single tube containing sucrose was presented, and mice were given 180 s to initiate licking. Once licking was recorded, mice were allowed 30 s access to the tube. At the conclusion of either the 30 s access or the 180 s limit, the shutter closed for 10 s. This repeated until each of the 8 tubes was presented 3 times.

During the remaining 3 d of training, the mice were given 30 min to initiate licking to one of 8 tubes of sucrose. However, unlike training day 2, the mice were exposed to a series of sucrose concentrations. The presented tube remained until the mice initiated licking, or the program ended. Once the mice began licking, they were given 10 s to lick, after which the trial concluded. The shutter closed for a 10-s ITI while a new bottle was chosen at random. At the conclusion of the ITI, the shutter opened.

Brief-access corn-oil training

A subset of the mice trained using the corn-oil method because our first studies (experiment 1) suggested that the KO mice were not responsive to sweet stimuli. Although all mice appeared trained and initiated trials, we were surprised by some of our findings (discussed later) and therefore, we switched to a fat training stimulus just to be sure that potential perceptual differences during training were not contributing to our findings. Mice were adapted to the test chamber and trained to drink from the sipper tubes for 7 consecutive days. During training, mice were 20-h water deprived. On day 1 through 4 of training, mice were presented with a single stationary bottle of water for 30 min, and the total number of licks to that bottle was recorded. In order to move forward in the training procedure, mice were required to lick a sufficient number of times (200 licks) at the stationary bottle, indicating that they were able to find the stimuli presented. On days 5 and 6 of training, mice were presented with a single stationary bottle of 4.5% corn-oil emulsion (w/v; 4.5% Mazola corn oil and 0.6% Tween 80 in tap water) and again were required to lick a sufficient number of times before moving on to the testing phase. On day 7, mice were trained in a brief-access taste test with water bottles. Animals were fed ad lib during training except in experiment 4 where animals were trained while food restricted. This is noted below in the description of those experiments.

Brief-access testing

During brief-access testing, animals were placed in the Davis rig as described during training but were allowed to complete as many trials as possible in 30 min. Each stimulus was presented in randomized blocks on Monday, Wednesday, and Friday in a single week. There is 1 exception to the schedule: in experiment 4 animals were tested before and after single-sugar access training and this procedure is described in more detail later.

Data analysis

We used lick correction factors to standardize for individual differences in lick rate and/or water need. For example, an animal that drinks a lot of water likely will also lick more sugar than an animal that drinks little water. This increase in sugar licking is likely not related to taste-driven behavior but is due to differences in motivation. In cases where licks to water are high, we use a lick ratio. Lick ratios are calculated by dividing the average number of licks at each concentration by the average number of licks to water. In cases where licks to water are few, we used a lick score. Lick scores are calculated by subtracting the average number of licks to water from the average number of licks at each concentration. We saw high variation across our experiments on water licking (discussed later), so we had to choose between using a standardized correction throughout the paper or using the correction factor that best suited each experiment. We chose the latter as a misused lick ratio could be very misleading (e.g. an animal with 70 licks to sucrose but 1 to water would have a ratio of 70, while an animal with the same sucrose behavior but 2 licks to water would have a ratio of 35. This second animal did drink twice as much water, but it is misleading to imply they were half as interested in sucrose). Likewise, when water licking is quite high, lick scores can be difficult to understand (e.g. negative values). Therefore, lick ratios were calculated for experiments when the animals were water deprived and when licks to water per trial exceeded 20 licks. Lick scores were calculated for experiments with fewer than 20 licks to water. All corrections and statistical analyses were done within experiments, so there are no comparisons presented across lick scores and lick ratios.

Licking behaviors were compared by repeated measures ANOVA with genotype as a between factors variable and concentration as repeated measures within factor variable. ANOVAs were conducted using Systat 13.

Experiments

Experiment 1: the role of PLCβ3 and TRPM4 in taste responding

PLCβ3 KO (n = 8), IP_3R3 KO (n = 8), TRPM4 KO (n = 10), TRPM5 KO (n = 10) mice, and C57Bl/6 (n = 7) mice were exposed to a series of sugars and maltodextrins in the Davis rig to determine the relative contributions of each of these signaling pathways to behavioral responsivity to saccharides. Animals were trained using the sugar-training method and were tested while 22-h water deprived. Data were calculated into lick scores.

Stimuli

All training and testing stimuli were prepared daily with tap water and presented in random order at room temperature. Mice were tested on sucrose (0.0625, 0.125, 0.25, 0.5, and 1 M), fructose (0.0625, 0.125, 0.25, 0.5, and 1 M), glucose (0.125, 0.25, 0.5, 1, and 2 M), maltose (0.0625, 0.125, 0.25, 0.5, and 1 M), and 3 differently sized maltodextrins (dextrose equivalent [DE]: 16.5 to 19.5, Sigma-Aldrich #419699; DE: 13 to 17, Sigma-Aldrich #419680; DE: 4 to 7, Sigma-Aldrich #419672; 1.25%, 2.5%, 5%, 10%, and 20%), which were tested in this order, consecutively, with 1 tastant presented each consecutive week. Sucrose was commercial grade (Walmart). All other chemicals were reagent grade from Sigma-Aldrich (St. Louis, MO). It is important to note that commercially available maltodextrins are not characterized in terms of chain length, so we do not know what chain lengths of maltodextrin are in each product. A general rule of thumb dictates that DE × chain length = 120, but this is not highly precise. Therefore, each maltodextrin product tested will overlap such that the largest DE in 1 stimulus solution may appear as one of the smaller DEs in the next stimulus e.g. one of the maltose stimuli is made up of DE units 13 to 17, while another is made up of 16 to 19 DEs.

Experiment 2: are TRPM4 and TRPM5 required for saccharide taste?

To follow up on the findings of experiment 1 (TRPM4 and TRPM5 lick responses were attenuated but not abolished—see results), TRPM4/5 DKO mice ($n = 8$) and C57Bl/6 WT mice ($n = 7$) were tested on a series of sugar and maltodextrin solutions. Brief-access training and testing, stimuli, and data analysis are identical to those from experiment 1. Mice were trained using the sugar-training method. Data are presented as lick scores.

Stimuli

All training and testing stimuli were prepared daily with tap water and presented at room temperature. Mice were tested with sucrose (0.0625, 0.125, 0.25, 0.5, and 1 M), glucose (0.125, 0.25, 0.5, 1, and 2 M), maltose (1, 0.5, 0.25, 0.125, 0.0625, 0.125, 0.25, 0.5, and 1 M), and maltodextrin (Maltrin QD DE ~15 to 19.9; 1.25%, 2.5%, 5%, 10%, and 20%). Maltrin QD was commercial grade (Grain Processing Corporation).

Experiment 3: replicating sucrose and glucose curves in PLCβ3 KO mice

We were surprised by the findings in experiment 1 (all sweet stimuli except glucose were blunted by the loss of PLCβ3—see results). The goal of experiment 3 was to confirm our findings from experiment 1, that PLCβ3 KO mice could still respond to glucose but not sucrose and to extend them to determine if these mice treated non-caloric sweeteners in the same way as sugars. In our previous work, we saw no difference in responding to sucrose and Ace-K between C57Bl/6 WT mice and the mixed genetic background mouse the PLCβ3 KO was bred into (Dutta Banik et al. 2020); therefore, experiment 1 relied on the C57Bl/6 WT as a control group. The main goal of experiment 3 was to rerun the glucose and sucrose stimuli using PLCβ3 KOs and the mixed genetic background mouse that PLCβ3 was knocked out of (MGB WT) as a control to confirm our finding was not due to differences in the background strain. We also included the C57Bl/6 and the IP_3R3 KO. Additionally, we included a group of artificial sweeteners in this cohort, to estimate whether the glucose finding was unique or if other sweet stimuli act independently of PLCβ3.

Brief-access training and testing

Mice (MGB WT: $n = 11$, PLCβ3 KO: $n = 12$, C57Bl/6: $n = 6$, IP_3R3 KO: $n = 8$) were trained using the corn-oil training paradigm under chronic food- and acute water-deprived conditions. Data were calculated as lick scores.

Stimuli

All training and testing stimuli were prepared daily with tap water and presented at room temperature. Mice were tested on sucrose (0.0625, 0.125, 0.25, 0.5, and 1 M), glucose (0.125, 0.25, 0.5, 1, and 2 M), and 3 artificial sweeteners: SC45647 (0.01, 0.03, 0.1, 0.3, and 1 mM), saccharin (0.5, 1, 5, 10, and 50 mM), and sucralose (1.25, 2.5, 5, 10, and 20 mM), which were tested in this order, consecutively, with 1 tastant presented each consecutive week. Saccharin and sucralose were reagent grade and purchased from Sigma-Aldrich (St. Louis, MO), SC45647 is described in Bachmanov et al. (2001).

Experiment 4: does IP_3R3 contribute to glucose/fructose differentiation?

Experiments 1 and 3 suggested that the PLCβ3 KO mice responded normally to glucose but the IP_3R3 KO mice did not (see results). Licking to stimuli in the brief-access taste test is affected by detection of the stimulus and the hedonic value of the stimulus. Therefore, changing how pleasant a sugar tastes would reduce licking in the same way that altering the concentration would. We decided to follow up the reduced responding in IP_3R3 KO mice with a more complex task where we ask the animal to differentiate 2 sugars. Glucose/fructose differentiation is an additional method to ask if an animal in a state of need can determine which solution contains metabolically needed glucose instead of the less valuable fructose (Schier and Spector 2016). In order to test whether IP_3R3 contributed to glucose/fructose differentiation, IP_3R3 KO and B6 WT mice received extensive exposure to various concentrations of glucose and fructose. Baseline and conditioned taste-guided responses were measured before and after sugar exposure in brief-access taste tests.

Stimuli

Reagent-grade glucose (0.316, 0.56, and 1.1 M) and fructose (0.316, 0.56, and 1.1 M) (Sigma-Aldrich) were prepared fresh with tap water as needed.

Food and water deprivation procedures

Mice were trained in the Davis rig using the corn-oil training paradigm while food and water deprived. Four types of deprivation states were used to motivate mice to lick from the sipper tube and initiate trials in the single-access exposure phase and brief-access tests: acute and chronic water deprivation and acute and chronic partial food deprivation. During acute water deprivation, home cage water bottles were removed ~22 h prior to testing and returned ~30 min after the testing session ends. During chronic water deprivation, home cage water bottles were removed, and fluid access was limited to the 30-min training session on each day. Mice

that fell below 85% of their ad libitum body weight were provided supplemental water (1 to 2 mL) in the home cage after the daily session. Acute food deprivation consisted of removing chow from the home cage ~22 h before the test session and returning chow ~30 min after the test session. Chronic partial food deprivation consisted of gradually reducing mice to 85% of their ad libitum body weight through daily chow rationing.

Single- and brief-access testing

During the brief-access taste tests, mice were presented with 6 sugar stimuli (0.316, 0.56, and 1.1 M glucose and fructose) and water in randomized blocks of 10-s trials (7.5-s ITI) in a 30-min session starting with 0.56 M glucose. This paradigm was run in 2 pretest series, once water deprived and once food restricted (acute). During the water-deprived pretest, mice were chronically water deprived and water was returned ~30 min after the session. After a 2-d break with ad libitum food and water, mice were then food restricted for ~22 h for the second brief-access pretest. Both pretests used the same session parameters.

Following the 2 brief-access pretests, mice were given a 2-d break with ad libitum food and water and then began the single-bottle exposure phase. The single-bottle exposure provided the animals the opportunity to associate the orosensory properties of each sugar with its postoral consequences and was designed as four 6-d blocks. The animals were chronically water deprived for blocks 1 and 2 and chronically food deprived for blocks 3 and 4. During the 6-d blocks, mice received 30-min access to one of the stimuli concentrations used in the brief-access test (0.316, 0.56, and 1.1 M glucose and fructose) with each sugar concentration presented 1 time per block. Half of the mice started with 0.56 M glucose and the other half started with 0.56 M fructose (presentation order shown in Table 1). The order of sugar presentation was replicated from a previous paper (see Schier and Spector 2016). During the single-access exposure phase, the shutter of the Davis rig is opened at the beginning of the testing session and kept open for 30 min. After the first 2 blocks, mice were given a 2-d break with ad libitum food and water and then chow was removed to reduce mice to 85% of their ad libitum body weight through daily chow rationing. Once mice were at a reliable 85% (±3%) ad lib body weight, the single-access phase continued for 2 more blocks while food deprived. Chow rations were provided ~30 min after each session. At the conclusion of the single-bottle training, all mice were given a 2-d break with ad libitum food and water before brief-access testing resumed. The brief-access posttest was conducted while the mice were acutely food restricted and chow was returned ~30 min after testing.

Data analysis

Licking behavior is expressed as lick scores. For the single-access sugar phase, total licks were collapsed across concentration for each sugar and calculated as average total licks by sugar for each block. Total licks were compared by repeated measures ANOVA with genotype as a between factors

Table 1. Testing schedule for experiment 4.

Phase	Day	Deprivation state	Groups and stimuli	
			Gp. A (WT $n = 4$; KO $n = 4$)	Gp. B (WT $n = 5$; KO $n = 4$)
Single-access training	1 to 3	Chronic water	H_2O/corn oil	
Brief-access training	4 to 6	Chronic water	H_2O	
Brief-access pretests	7	Acute water	0.316, 0.56, and 1.1 M glucose and fructose, H_2O	
	8 to 9	Ad lib food and water		
	10	Acute food	0.316, 0.56, and 1.1 M glucose and fructose, H_2O	
Break	11 to 12	Ad lib food and water		
Single-access exposure phase	13	Chronic water	0.56 M G	0.56 M F
	14	Chronic water	1.1 M F	1.1 M G
	15	Chronic water	0.316 M F	0.316 M G
	16	Chronic water	1.1 M G	1.1 M F
	17	Chronic water	0.56 M F	0.56 M G
	18	Chronic water	0.316 M G	0.316 M F
	19 to 24	Chronic water	…	…
	25 to 34	Chow rationing		
	35	Chronic food	0.56 M G	0.56 M F
	36	Chronic food	1.1 M F	1.1 M G
	37	Chronic food	0.316 M F	0.316 M G
	38	Chronic food	1.1 M G	1.1 M F
	39	Chronic food	0.56 M F	0.56 M G
	40	Chronic food	0.316 M G	0.316 M F
	41 to 46	Chronic food	…	…
Break	47 to 48	Ad lib food and water		
Brief-access posttest	49	Acute food	0.316, 0.56, and 1.1 M glucose and fructose, H_2O	

variable and sugar and block (i.e. time) as repeated measures within factors variables.

Results

Experiment 1: taste-driven licking to glucose is unaltered after genetic deletion of PLCβ3; licking to all other saccharides were modified

Our previous work suggested that TRPM4, TRPM5, IP_3R3, and PLCβ3 each contributed to normal taste-driven licking for sucrose. In fact, loss of IP_3R3 and PLCβ3 completely eliminated taste-driven licking for this representative sugar (Dutta Banik et al. 2020). Here, we tested mice in a water-restricted state to determine whether key signaling proteins are also recruited to drive licking responses to other saccharides in single KO mice, compared with B6 WT control mice. Lick scores for each taste stimulus are shown in Table 2, and plotted for glucose (Fig. 1A), sucrose (Fig. 1B), and maltodextrin DE 13 to 17 (Fig. 2).

Monosaccharides

Taste-driven licking for glucose was significantly different among the genotypes tested. Consistent with previous work, TRPM4 KO displayed attenuated concentration-dependent licking for glucose, compared with B6 WT mice. A similar deficit was observed in TRPM5 KO mice. IP_3R3 KO showed no responsiveness to glucose. In contrast, PLCβ3 KO licked in a normal concentration-dependent fashion for this sugar, at levels that were indistinguishable from that of the B6 WT controls (Fig 1A). Genotype-specific statistics are summarized in Table 2.

On fructose tests, TRPM4 KO and TRPM5 KO mice demonstrated significantly attenuated concentration-dependent licking, while IP_3R3 KO and PLCβ3 KO mice showed no concentration-dependent increase in lick responses.

Disaccharides

Taste-driven licking for sucrose largely replicated our published findings (Dutta Banik et al. 2018, 2020). TRPM4 KO, TRPM5 KO, and PLCβ3 KO mice had attenuated lick responses to sucrose, while IP_3R3 KO showed virtually no concentration-dependent responsiveness (Fig. 1B). However, it is notable that we had extremely low intake on the sucrose trials in all groups (even to water) despite the animals being in a water-deprived state. TRPM4 KO, TRPM5 KO, PLCβ3 KO,

Table 2. Summary of ANOVAs comparing average lick scores (± SEM) between all mouse lines.

Stimulus	Mouse line	0.125 M	0.25 M	0.5 M	1 M	2 M	Genotype	Concentration	Geno × conc
Glucose	B6 WT	0.14 ± 2.86	0.18 ± 3.15	11.30 ± 6.23	16.75 ± 4.68	27.58 ± 2.87	$P = 0.009^*$	$P < 0.001^*$	$P < 0.001^*$
	IP_3R3 KO	0.63 ± 2.30	−0.01 ± 3.83	−2.23 ± 3.03	−2.32 ± 3.70	−0.95 ± 3.73			[a]
	PLCβ3 KO	1.45 ± 4.00	13.48 ± 4.55	14.64 ± 6.91	22.83 ± 6.85	34.26 ± 7.69			
	TRPM4 KO	1.45 ± 2.21	1.67 ± 4.59	10.08 ± 4.48	10.48 ± 3.19	22.81 ± 3.35			[a]
	TRPM5 KO	3.44 ± 2.22	2.78 ± 1.32	1.34 ± 2.65	10.63 ± 3.39	20.96 ± 5.58			[a]
Stimulus	**Mouse line**	**0.0625 M**	**0.125 M**	**0.25 M**	**0.5 M**	**1 M**	**Genotype**	**Concentration**	**Geno × conc**
Fructose	B6 WT	−1.2 ± 1.29	10.15 ± 3.44	19.36 ± 3.82	19.73 ± 5.18	23.43 ± 4.21	$P = 0.001^*$	$P < 0.001^*$	$P < 0.001^*$
	IP_3R3 KO	−5.76 ± 3.89	−1.07 ± 4.58	−2.73 ± 5.90	−8.84 ± 4.86	−8.43 ± 4.35			[a]
	PLCβ3 KO	2.29 ± 3.41	3.02 ± 4.82	−3.65 ± 3.89	−7.42 ± 4.53	−1.21 ± 6.69			[a]
	TRPM4 KO	3.27 ± 3.67	6.62 ± 6.50	4.54 ± 6.97	3.44 ± 4.59	7.05 ± 5.08			[a]
	TRPM5 KO	4.22 ± 5.73	4.77 ± 4.52	9.09 ± 6.45	10.02 ± 2.19	9.00 ± 6.63			[a]
Stimulus	**Mouse line**	**0.0625 M**	**0.125 M**	**0.25 M**	**0.5 M**	**1 M**	**Genotype**	**Concentration**	**Geno × conc**
Sucrose	B6 WT	3.51 ± 1.46	5.39 ± 2.13	8.27 ± 2.90	8.75 ± 2.43	15.28 ± 1.54	$P = 0.006^*$	$P = 0.004^*$	$P = 0.077$
	IP_3R3 KO	−0.02 ± 2.73	1.00 ± 1.72	1.08 ± 1.40	0.48 ± 2.01	0.90 ± 1.16			[a]
	PLCβ3 KO	0.94 ± 2.19	1.44 ± 1.41	1.78 ± 2.66	−0.85 ± 1.00	4.99 ± 1.93			[b]
	TRPM4 KO	1.41 ± 2.69	3.23 ± 2.17	2.65 ± 3.57	3.63 ± 2.92	5.29 ± 1.22			[a]
	TRPM5 KO	1.76 ± 1.96	3.72 ± 2.14	3.88 ± 2.72	3.54 ± 6.95	6.57 ± 3.00			
Stimulus	**Mouse line**	**0.01 M**	**0.03 M**	**0.1 M**	**0.3 M**	**1 M**	**Genotype**	**Concentration**	**Geno × conc**
Maltose	B6 WT	0.21 ± 1.93	1.38 ± 1.20	2.10 ± 4.91	17.88 ± 6.70	19.86 ± 4.27	$P = 0.018^*$	$P < 0.001^*$	$P < 0.001^*$
	IP_3R3 KO	0.57 ± 3.05	0.60 ± 4.26	2.60 ± 7.09	−1.15 ± 8.67	−4.39 ± 4.11			[a]
	PLCβ3 KO	9.25 ± 4.42	−0.28 ± 7.31	7.32 ± 4.93	0.83 ± 2.85	2.23 ± 6.44			[a]
	TRPM4 KO	1.71 ± 4.03	−0.73 ± 2.70	3.61 ± 5.51	6.77 ± 2.55	10.40 ± 4.65			
	TRPM5 KO	0.29 ± 4.03	−1.98 ± 3.97	0.89 ± 4.62	6.39 ± 2.46	7.95 ± 1.98			[a]

Analyses were conducted with genotype as the between-subjects comparison and concentration as the within-subjects comparison.
[a]A significant geno × conc interaction compared with B6 WT control group (*P*s < 0.05).
[b]A trend in geno × conc interaction compared with B6 WT control group (*P* = 0.09).
*Ps < 0.05.

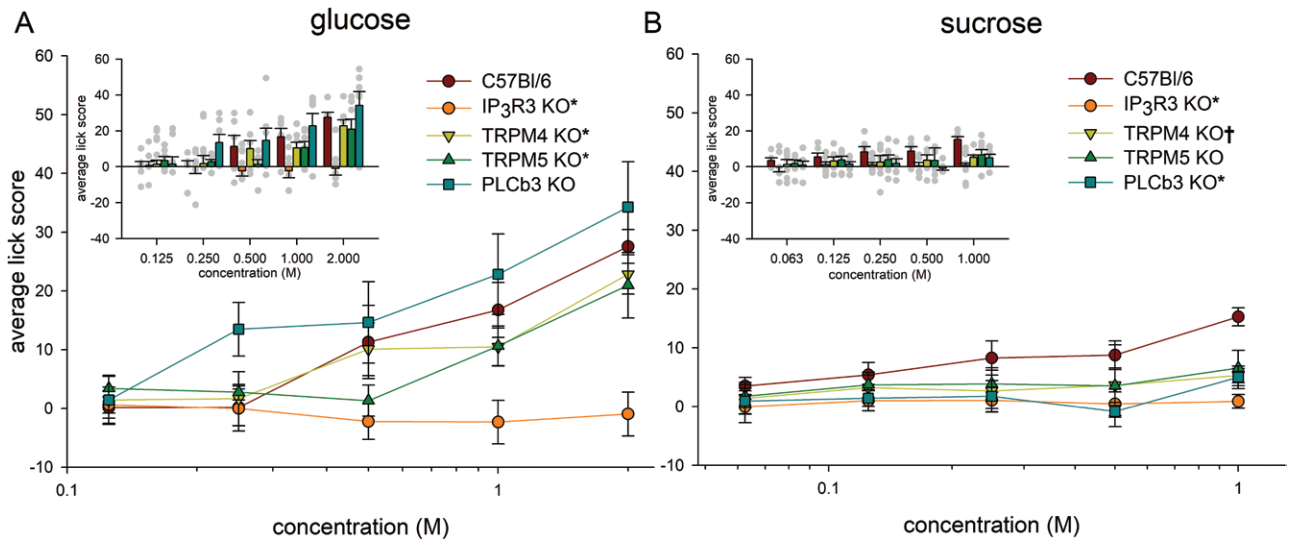

Fig. 1. Graph A (left) represents taste-driven licking to glucose while graph B (right) represents licking to sucrose. Data are average lick scores (± SEM) for glucose (A) and sucrose (B) between C57BL/6 WT (dark red circle), IP_3R3 KO (orange circle), TRPM4 KO (dark yellow upside-down triangle), TRPM5 KO (green triangle), and PLCβ3 KO (dark cyan square) mice under water-deprived conditions. Significance from C57BL/6 WT (*P*s < 0.05) is represented by an asterisk in the legend. Trending difference from C57BL/6 WT (*P*s < 0.09) is represented by a crossbar. Inset graphs represent average lick score data from host graphs in bar plot form with data points representing each individual animal's lick score across concentration. (A) IP_3R3 KO, TRPM4 KO, and TRPM5 KO but not PLCβ3 KO mice displayed blunted licking to glucose compared with B6 WT controls (*P*s < 0.05). (B) IP_3R3 KO, PLCβ3 KO, TRPM4, and TRPM5 KO mice showed a decrease in taste-driven licking to sucrose compared with WT control mice (*P*s < 0.05).

and IP_3R3 KO mice displayed attenuated licking responses to maltose, compared with the B6 WT mice.

Polysaccharides

We tested polysaccharides of 3 different size classes (small polysaccharide DE 16.5 to 19.5, intermediate polysaccharide DE 13 to 17, and large polysaccharide DE 4 to 7). TRPM4 KO and TRPM5 KO, PLCβ3 KO, and IP_3R3 KO substantially blunted or abolished licking responses to all maltodextrins compared with WT mice (Fig. 2 and Table 3).

Experiment 2: genetic deletion of both TRPM4 and TRPM5 eliminated taste-driven behavioral responsiveness to glucose-containing saccharides

Single KOs of the TRPM4 and TRPM5 genes substantially attenuated but did not completely eliminate taste-driven licking responses for simple and complex saccharides comprised of glucose. Therefore, we assessed whether genetic deletion of both genes was sufficient to prevent taste-driven licking for these nutrients. TRPM4/5 DKO mice did not elicit licks for any glucose or sucrose concentration, relative to water (Fig. 3A and B). Nor did these mice respond to maltose or maltodextrin (Table 4).

Experiment 3: PLCB3 KO mice lick normally for glucose, but not sucrose or select artificial sweeteners

The fact that PLCβ3 KO mice licked avidly for glucose, but not sucrose, was striking and unexpected. Equally surprising was the fact that IP_3R3 KO completely eliminated responding to glucose, as this intracellular receptor is a critical channel in Type II taste cell signaling, and previous work has suggested that glucose may engage Type II cells in a T1R-independent fashion (Glendinning et al. 2015; Schier et al. 2019). Therefore, we tested new cohorts of PLCβ3 KO and IP_3R3 KO, alongside their respective WT controls, for their taste-driven responsiveness to glucose, sucrose, and select low calorie sweeteners to assess whether these findings are similarly evident in a different motivational state (food restriction). Indeed, PLCβ3 KO mice licked robustly to glucose, but not at all for sucrose (Fig. 4). Conversely, IP_3R3 KO mice did not lick for glucose at any concentration tested and licked modestly for sucrose at the highest concentrations tested.

PLCβ3 KO and IP_3R3 KO mice also displayed weakened or no taste-driven licking behavior to all 3 artificial sweeteners compared with WT mice (Table 5).

Experiment 4: IP_3R3 is not necessary to acquire an attraction to glucose through ingestive experience

Previous work suggested that exposure to the orosensory and metabolic consequences of glucose and fructose bolsters the relative avidity for glucose over that of fructose in brief-access tests, and this does not require input from the T1R receptors (Schier and Spector 2016; Schier et al. 2019; Ascencio Gutierrez et al. 2022). Here, we assessed if the heightened avidity for glucose over fructose requires IP_3R3. In an initial brief-access test to measure relative taste-driven responsiveness to glucose and fructose, WT mice licked comparably for both sugars at each concentration (Fig. 5A). IP_3R3 KO mice licked more for fructose compared with glucose, though licking was generally subdued (Fig. 5B). During the longer-term exposure sessions, WT mice licked similar amounts for the 2 sugars, while water restricted, but selectively increased licking for glucose once shifted to chronic calorie restriction (Fig. 6A). IP_3R3 KO mice initially licked more for fructose while water restricted (block 1 only), and although this appeared to shift toward more glucose with chronic calorie restriction, the difference between sugars was not statistically significant (Fig. 6B). During the postexposure brief-access test, WT and IP_3R3 KO mice licked significantly more for glucose over fructose, but this difference was notably more modest in the KO mice (Fig. 5C and D).

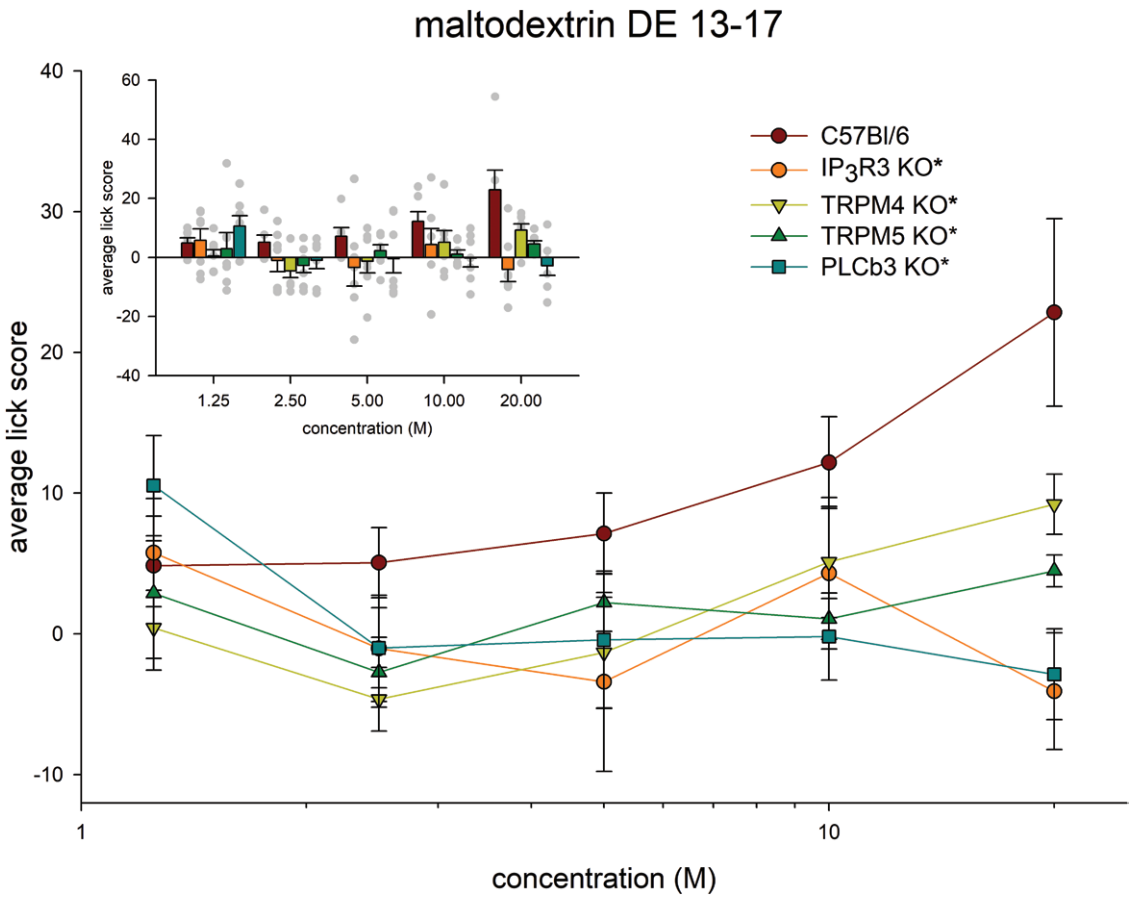

Fig. 2. Represents taste-driven licking to maltodextrin DE 13 to 17. Data are average lick scores (± SEM) for maltodextrin between C57BL/6 WT (dark red circle), IP_3R3 KO (orange circle), TRPM4 KO (dark yellow upside-down triangle), TRPM5 KO (green triangle), and PLCβ3 KO (dark cyan square) mice under water-deprived conditions. Significance from C57BL/6 WT (*P*s < 0.05) is represented by an asterisk in the legend. Inset graphs represent average lick score data from host graphs in bar plot form with data points representing each individual animal's lick score across concentration. IP_3R3 KO, TRPM4 KO, and TRPM5 KO, and PLCβ3 KO mice displayed blunted licking to glucose compared with B6 WT controls (*P*s < 0.05).

Latency to initiate licking for each sugar and concentration was analyzed to assess the potential contribution of olfactory cues to the acquired glucose versus fructose discrimination, as in Schier et al. (2019). Neither the WT nor the IP_3R3 KO mice exhibited differential latencies to lick for glucose versus fructose solutions in the initial brief-access test (Fig. 7A and B). As a group, the WT mice show longer latencies to lick for fructose at the 0.56 and 1.1 M concentrations in the postexposure test (Fig. 7C). IP_3R3 KO mice, on the other hand, continued to exhibit similar latencies for both sugars after exposure (Fig. 7D), though close inspection of the individual mouse data suggests that a couple of these mice may have used a cue at the highest sugar concentration.

General discussion

The traditional model of taste sensation proposes 2 cell types responsible for the 5 canonical taste qualities (Types II and III). The model specifically proposes that Type II cells are narrowly tuned receptor cells which respond to single taste qualities (bitter, sweet, or umami), however, we have recently begun exploring the role of a BR cell that responds to several stimulus qualities, including sour, bitter, sweet, and umami in normal taste responding. These cells use a PLCβ pathway that is different from the signaling pathway described in Type II cells (Dutta Banik et al. 2018). In our previous work, we demonstrated that the loss of PLCβ3 resulted in decreased taste-driven responses to Ace-K and sucrose compared with WT controls (Dutta Banik et al. 2020). We have also demonstrated a role for TRPM4 in BUS stimuli (Dutta Banik et al. 2018) broadening our understanding of taste-signaling pathways. Loss of either TRPM4 or TRPM5 greatly reduces responding to BUS stimuli (Dutta Banik et al. 2018). Although our previous work tested multiple classes of tastants (sweet, bitter, umami, salt, and sour), we tested relatively few example stimuli within each quality. Sweet stimuli are variable in size and type and can be differentiated, so here we extended our investigation to include a variety of monosaccharides, complex saccharides, and artificial sweeteners to determine the relative contribution of these signaling pathways to sweet and carbohydrate taste. We tested 5 lines of KO mice in brief-access taste tests and tested a range of sugars, the monosaccharides, glucose and fructose, and disaccharides, sucrose, and maltose; artificial sweeteners, saccharin, sucralose, and SC45647; and polysaccharides of multiple size classes. Because polysaccharides are comprised of chains of glucose molecules at varying lengths, we tested short (DE 16.5 to 19.5), intermediate (13 to 17), and long (DE 4 to 7) chain maltodextrins to also clarify whether chain length is important for taste-guided responses.

Table 3. Summary of ANOVAs comparing average lick scores (± SEM) between all mouse lines.

Stimulus	Mouse line	1.25%	2.5%	5%	10%	20%	Genotype	Concentration	Geno × conc
Maltodextrin DE 16.5 to 19.5	B6 WT	−2.22 ± 3.36	−2.56 ± 5.72	4.19 ± 8.11	−1.89 ± 8.35	28.01 ± 8.16	$P = 0.039^{*}$	$P = 0.001^{*}$	$P < 0.001^{*}$
	IP_3R3 KO	7.21 ± 3.73	2.87 ± 2.35	3.02 ± 2.98	−2.80 ± 3.91	−0.12 ± 2.17			[a]
	PLCβ3 KO	−3.72 ± 4.10	−1.30 ± 4.28	4.77 ± 4.66	2.38 ± 4.81	8.25 ± 6.39			[a]
	TRPM4 KO	2.85 ± 3.67	−2.44 ± 2.44	10.20 ± 4.52	6.72 ± 6.03	14.70 ± 6.34			[a]
	TRPM5 KO	3.43 ± 2.40	1.32 ± 4.03	1.51 ± 4.92	−0.15 ± 2.60	4.26 ± 6.19			[a]
Stimulus	**Mouse line**	**1.25%**	**2.5%**	**5%**	**10%**	**20%**	**Genotype**	**Concentration**	**Geno × conc**
Maltodextrin DE 13 to 17	B6 WT	4.86 ± 1.75	5.07 ± −1.49	7.13 ± 2.88	12.17 ± 3.26	22.84 ± 6.66	$P = 0.014^{*}$	$P = 0.009^{*}$	$P < 0.001^{*}$
	IP_3R3 KO	5.77 ± 3.84	−1.03 ± 3.77	−3.40 ± 6.35	4.31 ± 5.38	−4.07 ± 3.15			[a]
	PLCβ3 KO	0.42 ± 2.17	−4.64 ± 2.27	−1.33 ± 3.93	5.10 ± 2.95	9.21 ± 2.13			[a]
	TRPM4 KO	2.90 ± 5.47	−2.73 ± 2.49	2.24 ± 2.05	1.07 ± 2.45	4.48 ± 1.13			[a]
	TRPM5 KO	10.53 ± 3.56	−0.99 ± 2.84	−0.42 ± 4.87	−0.18 ± 3.09	−2.87 ± 3.23			[a]
Stimulus	**Mouse line**	**1.25%**	**2.5%**	**5%**	**10%**	**20%**	**Genotype**	**Concentration**	**Geno × conc**
Maltodextrin DE 4 to 7	B6 WT	8.39 ± 5.64	6.88 ± 3.74	9.90 ± 4.19	22.02 ± 6.78	34.34 ± 6.91	$P = 0.047^{*}$	$P < 0.001^{*}$	$P < 0.001^{*}$
	IP_3R3 KO	4.64 ± 6.10	−1.55 ± 7.66	0.28 ± 6.70	−4.08 ± 8.55	4.98 ± 3.94			[a]
	PLCβ3 KO	−0.85 ± 3.96	−2.04 ± 2.17	2.75 ± 3.33	5.50 ± 2.93	9.29 ± 6.93			[a]
	TRPM4 KO	5.60 ± 2.78	−3.38 ± 4.85	2.62 ± 5.55	8.35 ± 4.42	16.61 ± 6.25			[a]
	TRPM5 KO	3.60 ± 5.93	−5.82 ± 2.94	−6.21 ± 6.76	−3.91 ± 0.87	−2.70 ± 3.67			[a]

Analyses were conducted with genotype as the between-subjects comparison and sugar as the within-subjects comparison.
[a]A significant geno × conc interaction compared with B6 WT controls (*P*s < 0.05).
*Ps < 0.05.

Glucose emerges as distinct from other saccharides and sweeteners

Loss of TRPM4/5 or IP_3R3 function considerably attenuated or even abolished taste-driven licking for all stimuli tested. In contrast, PLCβ3 contributes to the behavioral responsiveness to many saccharides and sweeteners but is not necessary for glucose. We found that loss of PLCβ3 resulted in blunted licking response to fructose, sucrose, and maltose, but not glucose. This glucose effect was not dependent on water restriction, as licking motivated by food restriction recapitulated the robust responsiveness for glucose in PLCβ3 KO mice which further provides evidence of the unique qualities of glucose as a sweet stimulus. These data agree with numerous studies, which have highlighted glucose as distinct from other sugars (Glendinning et al. 2015, 2017; Schier and Spector 2016; Schier et al. 2019; Ascencio Gutierrez et al. 2022; Chometton et al. 2022). Recent studies have proposed various T1R2/T1R3-independent signaling mechanisms including the sodium glucose linked transporter 1/3 (Yasumatsu et al. 2020), glucokinase (Chometton et al. 2022), and/or a K_{ATP} channel (Glendinning et al. 2017); however, if and how these proteins interact with one another and whether the glucose-generated signal still relies on the Type II cell require further investigation. Future work could make use of non-metabolizable glucose analogs such as MDG (alpha-methyl-D-glucopyranoside) in combination with PLCβ3 KO mice to further elucidate glucose's unique signaling pathway(s).

Polysaccharides are comprised of chains of glucose molecules that are believed to be cleaved into glucose, disaccharides, and trisaccharides by amylase and other α-glucosidases in the oral cavity (Woolnough et al. 2010; Sukumaran et al. 2016; Glendinning et al. 2017). Free glucose can elicit a cephalic-phase insulin release (CPIR) in mice that do not have an intact sweet receptor (Glendinning et al. 2015)

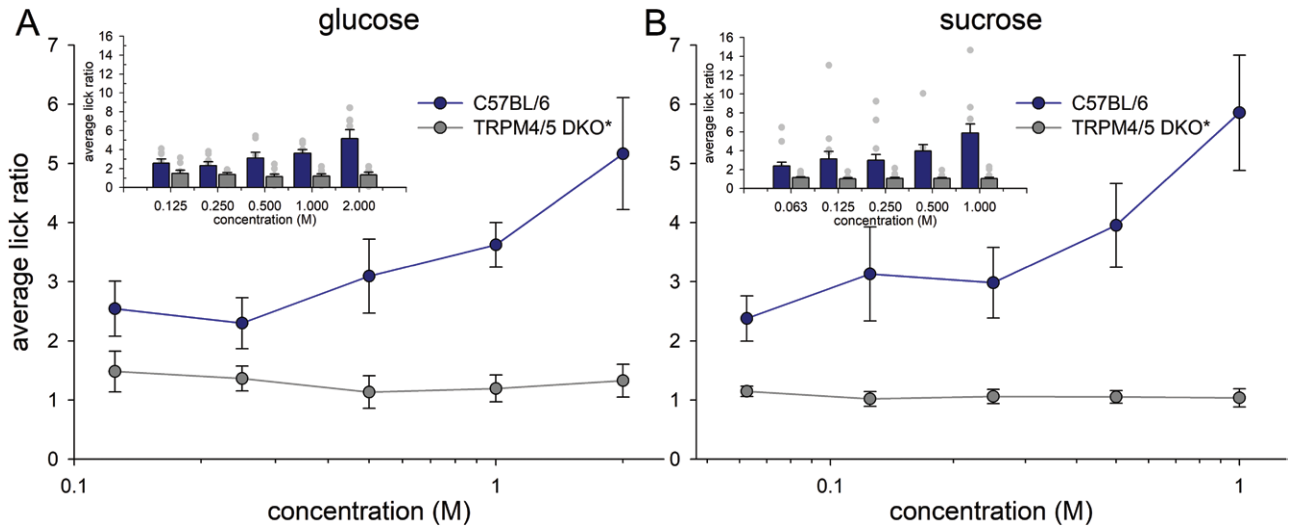

Fig. 3. Graph A (left) represents taste-driven licking to glucose while graph B (right) represents licking to sucrose in a TRPM4/5 DKO and B6 WT mice. Data are average lick ratios (± SEM) for glucose (A) and sucrose (B) between TRPM4/5 DKO (gray circle) and B6 WT mice (dark blue circle) under water-deprived conditions. Significance from C57BL/6 WT is represented by an asterisk in the legend. Inset graphs represent average lick ratio data from host graphs in bar plot form with data points representing each individual animal's lick ratio across concentration. TRPM4/5 DKO mice decrease their taste-driven responses to both glucose (A) and sucrose (B) compared with WT controls (*P*s < 0.05).

Table 4. Summary of ANOVAs comparing average lick ratios (± SEM).

Stimulus	Mouse line	0.125 M	0.25 M	0.5 M	1 M	2 M	Genotype	Concentration	Geno × conc
Glucose	B6 WT	2.54 ± 0.45	2.30 ± 0.43	3.09 ± 0.62	3.62 ± 0.38	5.16 ± 0.95	$P = 0.002^*$	$P = 0.029^*$	$P = 0.014^*$
	TRPM4/5 DKO	1.48 ± 0.35	1.36 ± 0.21	1.14 ± 0.27	1.20 ± 0.23	1.33 ± 0.28			
Stimulus	**Mouse line**	**0.0625 M**	**0.125 M**	**0.25 M**	**0.5 M**	**1 M**	**Genotype**	**Concentration**	**Geno × conc**
Sucrose	B6 WT	2.38 ± 0.38	3.13 ± 0.80	2.98 ± 0.60	3.95 ± 0.71	5.86 ± 0.97	$P < 0.001^*$	$P < 0.001^*$	$P < 0.001^*$
	TRPM4/5 DKO	1.15 ± 0.09	1.02 ± 0.12	1.06 ± 0.12	1.05 ± 0.11	1.04 ± 0.15			
Stimulus	**Mouse line**	**0.0625 M**	**0.125 M**	**0.25 M**	**0.5 M**	**1 M**	**Genotype**	**Concentration**	**Geno × conc**
Maltose	B6 WT	1.62 ± 0.38	2.01 ± 0.30	1.57 ± 0.43	3.06 ± 0.73	4.72 ± 1.09	$P = 0.018^*$	$P < 0.001^*$	$P < 0.001^*$
	TRPM4/5 DKO	1.21 ± 0.14	1.45 ± 0.24	1.27 ± 0.20	1.67 ± 0.23	1.12 ± 0.14			
Stimulus	**Mouse line**	**1.25%**	**2.5%**	**5%**	**10%**	**20%**	**Genotype**	**Concentration**	**Geno × conc**
Maltrin QD	B6 WT	1.62 ± 0.38	2.01 ± 0.30	1.57 ± 0.43	3.06 ± 0.73	4.72 ± 1.09	$P = 0.018^*$	$P < 0.001^*$	$P < 0.001^*$
	TRPM4/5 DKO	1.21 ± 0.14	1.45 ± 0.24	1.27 ± 0.20	1.67 ± 0.23	1.12 ± 0.14			

Analyses were conducted with genotype as the between-subjects comparison and sugar as the within-subjects comparison.
$^*P$s < 0.05.

suggesting that complex sugars such as sucrose, maltose, and maltodextrins can potentially be detected upon oral digestion through an unknown glucose sensing mechanism. However, although glucose responding was unchanged in the PLCβ3 KO, the mice did not respond to more complex saccharides, suggesting that these mice did not cleave sufficient amounts of free glucose from disaccharides and polysaccharides to engage this glucose detection system, at least within these brief-access trials and in a water-restricted state.

IP_3R3 is not necessary for the acquired preference for glucose

In the absence of IP_3R3, mice did not lick for glucose beyond that of water in brief-access tests, and displayed reduced licking responses for sucrose, even at high concentrations, in a food-deprived state. Consistent with this, it was notable that IP_3R3 KO mice responded more positively to fructose than glucose in pretraining testing. However, previous studies have shown that mice lick more for glucose than fructose in brief-access tests, when they have had the opportunity to experience the unique sensory and metabolic consequences of these 2 sugars (Schier and Spector 2016; Schier et al. 2019; Ascencio Gutierrez et al. 2022). While Chometton et al. (2022) showed that lingual glucokinase is involved in the expression of this acquired avidity for glucose in B6 WT mice, Glendinning et al. (2020) found that mice lacking the sulfonylurea receptor 1, associated with downstream K_{ATP}-mediated glucosensing, readily acquired the hedonic discrimination. Here, we found

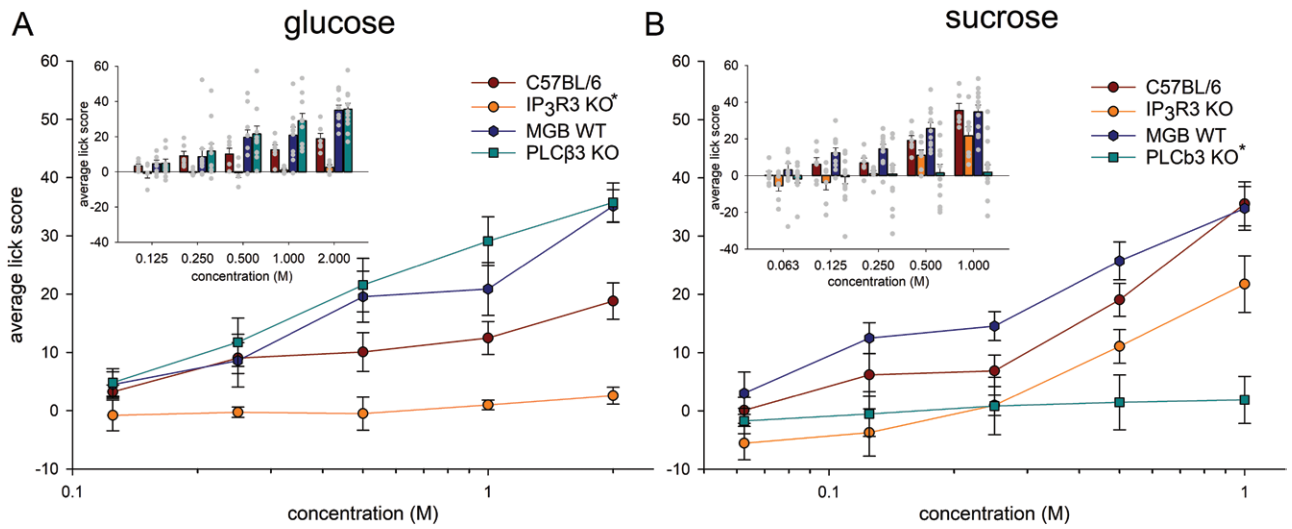

Fig. 4. Graph A (left) represents taste-driven licking to glucose while graph B (right) represents licking to sucrose in a new cohort of mice. Data are average lick scores (± SEM) for glucose (A) and sucrose (B) between C57BL/6 (dark red circle), MGB WT (dark blue circle), IP_3R3 KO (orange circle), and PLCβ3 KO (dark cyan square) mice under water-deprived (glucose) and food and water-deprived (sucrose) conditions. Significance from C57BL/6 WT is represented by an asterisk in the legend. Inset graphs represent average lick score data from host graphs in bar plot form with data points representing each individual animal's lick score across concentration. (A) IP_3R3 KO (orange) mice decreased their taste-driven response to glucose compared with B6 WT (dark red) controls ($P < 0.05$). Both PLCβ3 KO (dark cyan) and MGB WT (dark blue) mice showed a concentration-dependent increase in licking to glucose. (B) PLCβ3 KO mice decreased licking behavior to sucrose compared with PLCβ3 WT mice. Unlike experiment 1a, IP_3R3 KO mice show a blunted concentration-dependent licking response to sucrose compared with B6 WT mice.

that although IP_3R3 contributes to the *brief-access* licking response to glucose, it is not necessary to generate the licking responses for glucose following the extensive ingestive experience with the 2 sugars. This aligns with several studies that showed that mice lacking TRPM5, which also contributes to signal transduction in Type II cells, come to preferentially respond to glucose (Zukerman et al. 2013; Sclafani and Ackroff 2015; Sclafani et al. 2020), including in 10-s trial brief-access taste tests (Ascencio Gutierrez et al. 2022), but only after extensive ingestive experience with both sugars. The results suggest that other sensory pathways must be recruited through this type of sugar exposure to enable rapid glucose discrimination. Previous studies suggested that under certain conditions, mice may use non-taste cues, such as odors, to discriminate glucose from fructose (Schier et al. 2019; Glendinning et al. 2020). Although we did not investigate this directly, close inspection of the data revealed that WT mice appeared to initiate glucose trials with a shorter latency than fructose trials, especially at the highest concentration in the postexposure brief-access test, suggesting they may have been using an olfactory cue. IP_3R3 KO mice, on the other hand, were not generally relying on such cues to initiate licking to the 2 different sugars.

That IP_3R3 KO mice do not initially respond in brief-access tests to glucose or fructose but can learn to differentiate glucose from fructose in an acquired discrimination test highlights the need for multiple types of tests in exploring the importance of these signaling components. Brief-access licking is informed heavily by the hedonic evaluation of the stimulus and motivation to consume it, and therefore changing either of those factors can influence the results of the test. For example, considering only the brief-access licking suggests that IP_3R3 KO mice are unable to sense glucose and fructose, while the acquired discrimination task suggests that IP_3R3 KO mice are capable of differentiating based on some sensory feature(s). Further tests could benefit from separating evaluation of the taste quality or intensity from hedonic evaluation of the stimulus.

TRPM4 and TRPM5 contribute to saccharide and sweet sensing

Loss of TRPM4 or 5 blunted, but did not eliminate, taste-driven concentration-dependent licking for glucose, fructose, sucrose, maltose, and maltodextrins tested in a water-restricted state. To further assess if TRPM5 and TRPM4 are both required to drive brief-access licking behaviors for various glucose-yielding saccharides, we offered TRPM4/5 DKO glucose, sucrose, maltose, and Maltrin in separate brief-access taste tests. DKO mice did not show concentration-dependent increases in lick ratio for any stimulus; instead, all were treated as if they were water. This agrees with our previous findings that indicate the knockdown of both channels completely abolishes taste-guided response to sweeteners and other saccharides. TRPM5 is only expressed in the Type II cells (Liman 2007). However, the distribution of TRPM4 is not as well defined. TRPM4 has been described in both Type II and III (acid responsive) cells using live cell imaging (Dutta Banik et al. 2018). These studies did not test whether the TRPM4-containing Type III cell was a PLCβ3 containing BR cell but TRPM4 is required for normal signaling in BR TRCs so its effects in these cells may be contributing as well (Dutta Banik and Medler 2023). These data suggest that these channels are both important to contribute to normal responding across a wide array of sweet and maltodextrin stimuli.

What can we learn about the maltodextrin receptor system?

Previous research has suggested that Type II taste cells are responsible for maltodextrin taste (Zukerman et al. 2013) although the identity of the receptor remains elusive. Here, we

Table 5. Summary of ANOVAs comparing average lick scores (± SEM) between all mouse lines.

Stimulus	Mouse line	0.125 M	0.25 M	0.5 M	1 M	2 M	Genotype	Concentration	Geno × conc
Glucose	B6 WT	3.26 ± 1.17	9.05 ± 2.64	10.09 ± 3.31	12.49 ± 2.82	18.82 ± 3.12	$P < 0.001^*$	$P < 0.001^*$	$P < 0.001^*$
	IP_3R3 KO	−0.80 ± 2.63	−0.26 ± 0.89	−0.50 ± 2.85	0.99 ± 0.84	2.58 ± 1.48			
	MGB WT	4.51 ± 2.20	8.61 ± 4.52	19.58 ± 4.37	20.90 ± 4.53	35.12 ± 2.81			
	PLCβ3 KO	4.86 ± 2.37	11.76 ± 4.14	21.57 ± 4.61	29.11 ± 4.16	35.74 ± 3.33			
Stimulus	**Mouse line**	**0.0625 M**	**0.125 M**	**0.25 M**	**0.5 M**	**1 M**	**Genotype**	**Concentration**	**Geno × conc**
Sucrose	B6 WT	0.10 ± 2.23	6.20 ± 3.67	6.89 ± 2.69	19.06 ± 2.81	35.55 ± 3.76	$P < 0.001^*$	$P < 0.001^*$	$P < 0.001^*$
	IP_3R3 KO	−5.52 ± 2.87	−3.71 ± 0.98	0.98 ± 1.74	11.08 ± 2.89	21.77 ± 4.87			[a]
	MGB WT	3.06 ± 3.63	12.50 ± 2.65	14.58 ± 2.48	25.76 ± 3.25	34.76 ± 3.74			
	PLCβ3 KO	−1.72 ± 2.18	−0.51 ± 3.84	0.86 ± 4.93	1.48 ± 4.71	1.90 ± 4.05			[a]
Stimulus	**Mouse line**	**1.25 M**	**2.5 M**	**5 M**	**10 M**	**50 M**	**Genotype**	**Concentration**	**Geno × conc**
Sucralose	B6 WT	0.04 ± 0.81	5.23 ± 3.16	9.11 ± 3.66	11.29 ± 5.64	14.02 ± 5.19	$P = 0.001^*$	$P = 0.010^*$	$P = 0.260$
	IP_3R3 KO	0.32 ± 0.83	0.08 ± 1.41	2.42 ± 2.12	2.46 ± 1.71	2.38 ± 1.46			[b]
	MGB WT	7.73 ± 4.29	13.38 ± 4.62	18.95 ± 7.83	16.07 ± 5.40	21.46 ± 6.84			
	PLCβ3 KO	−0.61 ± 1.44	0.27 ± 1.08	−0.37 ± 0.98	−1.55 ± 2.22	−3.00 ± 3.13			[a]
Stimulus	**Mouse line**	**0.5 M**	**1 M**	**5 M**	**10 M**	**50 M**	**Genotype**	**Concentration**	**Geno × conc**
Saccharin	B6 WT	2.06 ± 1.49	6.14 ± 2.29	12.15 ± 3.23	11.90 ± 3.58	21.93 ± 6.29	$P = 0.001^*$	$P = 0.003^*$	$P = 0.020^*$
	IP_3R3 KO	1.68 ± 5.69	0.27 ± 3.52	0.11 ± 3.06	−3.95 ± 1.64	−0.52 ± 4.82			[a]
	MGB WT	5.83 ± 2.98	7.84 ± 2.08	15.64 ± 2.89	16.89 ± 4.61	31.17 ± 6.22			
	PLCβ3 KO	−0.39 ± 4.13	−3.39 ± 3.80	−0.49 ± 5.96	−3.08 ± 7.29	−3.66 ± 6.93			[a]
Stimulus	**Mouse line**	**0.01 M**	**0.03 M**	**0.1 M**	**0.3 M**	**1 M**	**Genotype**	**Concentration**	**Geno × conc**
SC45647	B6 WT	3.12 ± 0.80	4.65 ± 3.09	10.41 ± 3.52	17.23 ± 4.09	31.75 ± 3.98	$P < 0.001^*$	$P < 0.001^*$	$P < 0.001^*$
	IP_3R3 KO	0.86 ± 1.49	−1.45 ± 2.00	−1.17 ± 1.95	1.13 ± 2.12	1.03 ± 1.99			[a]
	MGB WT	8.90 ± 2.14	9.35 ± 2.43	23.20 ± 5.54	30.56 ± 3.23	44.09 ± 10.66			
	PLCβ3 KO	−0.08 ± 1.57	4.44 ± 3.07	0.78 ± 3.50	3.73 ± 2.15	0.57 ± 1.35			[a]

Analyses were conducted with genotype as the between-subjects comparison and sugar as the within-subjects comparison. *P* values represent ANOVA comparing all 4 mouse lines. Post hoc ANOVAs were conducted between B6 WT control mice and IP_3R3 KO mice, and MGB WT and PLCβ3 KO mice.
[a]A significant geno × conc interaction compared with B6 WT control group (*P*s < 0.05).
[b]A trend in geno × conc interaction compared with B6 WT control group (*P* = 0.09).
*Ps < 0.05.

aimed to extend these findings by asking whether the BR cell, through a PLCβ3 KO model, Type II taste cell, using IP_3R3 KO, and TRPM5 KO mouse lines, were necessary for maltodextrin taste. We found that mice lacking PLCβ3-dependent signaling were unable to normally respond to polysaccharides at various chain lengths, suggesting that the BR pathway is necessary for normal maltodextrin responding as it is for normal sweet responding. We further assessed whether TRPM4 and/or TRPM5, and IP_3R3 are necessary for normal maltodextrin responding. TRPM4 and TRPM5 single KOs show a blunted response to the 3 maltodextrins (DE 16.5 to 19.5, DE 13 to 17, and DE 4 to 7) and the TRPM4/5 DKO mice show almost no response. Likewise, IP_3R3 appears necessary for normal maltodextrin-related behavioral responses. These relationships mirror those we see with the sweet stimuli (except for glucose), which suggests that although detection of non-sugar carbohydrates is likely through an independent receptor, it is using the same signaling machinery that supports bitter, sweet, and umami taste responding. This agrees with published work suggesting that maltodextrin detection is likely reliant on the Type II cell (Glendinning et al. 2017) and additionally extends our previous work, demonstrating that the BR pathway is not only important for BUS brief-access responding, but maltodextrin responding as well.

Conclusion

Together these data suggest that various intracellular signaling proteins associated with Type II and BR cells are important to support normal taste-driven behavior for a wide range of sweet and carbohydrate stimuli in brief-access taste tests prior to any sugar experience. This is not the case for glucose, which does not appear to rely on PLCβ3 to initiate normal behavioral responding. This supports previous work suggesting that glucose has some unique qualities and may be using a distinct signaling pathway. These data also support previous work suggesting that maltodextrin signaling, although it may rely on an undefined receptor, likely uses some of the same intracellular signaling machinery as sweet stimuli. These findings provide a deeper understanding of the intracellular mechanisms in Type II and BR taste cells involved in sweet and carbohydrate

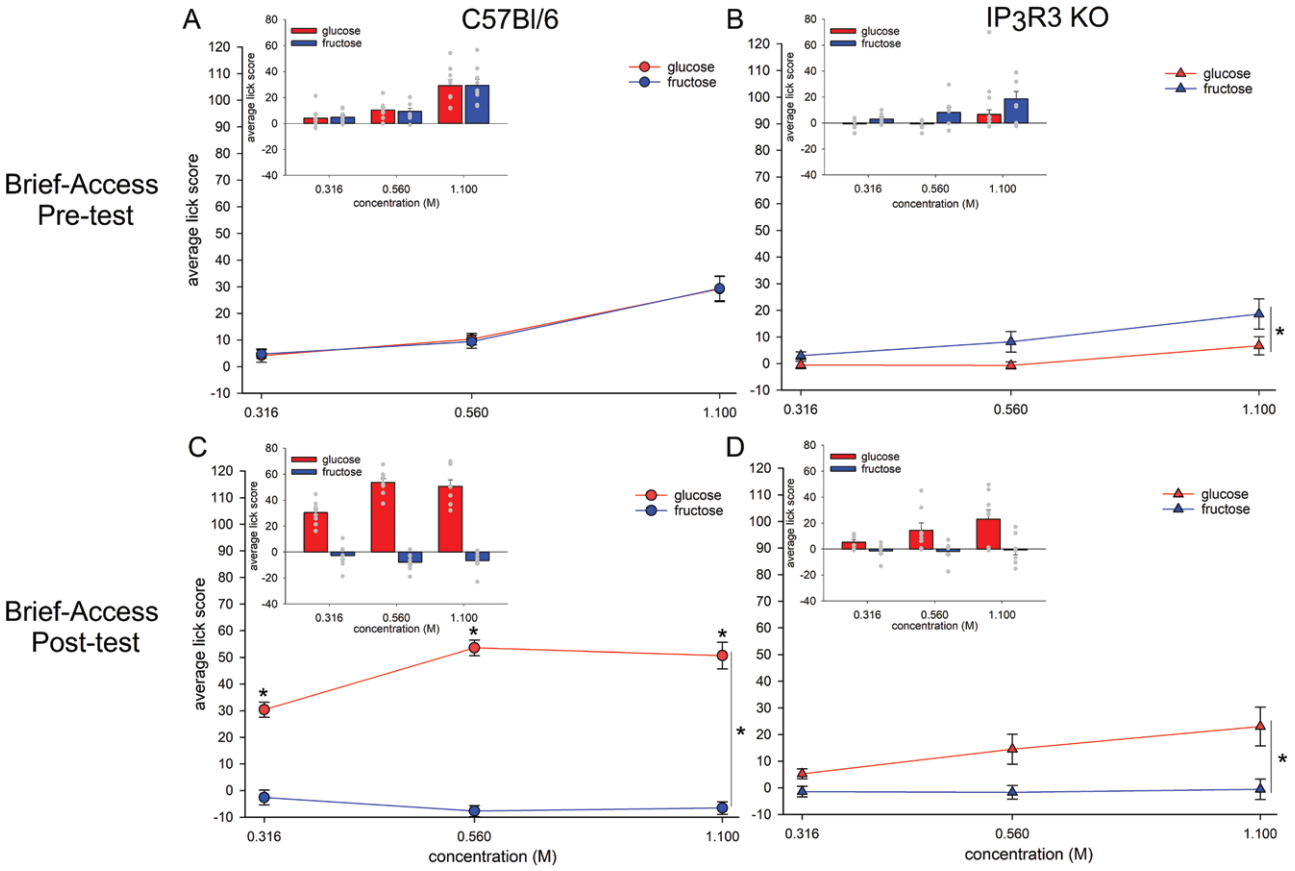

Fig. 5. (A–D) Brief-access licking behavior to glucose and fructose in B6 WT and IP_3R3 KO mice before and after sugar exposure. Data are average lick scores (± SEM) for glucose (red) and fructose (blue) in C57Bl/6 (circle) and IP_3R3 KO (triangle) mice before (pretest) and after (posttest) single-access sugar exposure. Inset graphs represent average lick score data from host graphs in bar plot form with data points representing each individual animal's lick score across concentration. (A, top left) B6 WT mice do not differ in their taste-guided response to glucose and fructose before sugar exposure. Inset graphs represent average lick score data in host graphs in bar plot form with data points representing each individual animal's lick score across concentration. (B, top right) IP_3R3 KO mice licked more for fructose over glucose in the brief-access pretest before sugar exposure ($P = 0.04$). (C, bottom left) B6 WT mice licked significantly more for all 3 concentrations of glucose over fructose in the brief-access posttest (after sugar exposure; $P < 0.001$). (D, bottom right) IP_3R3 KO mice licked significantly more for glucose over fructose overall in the brief-access posttest after sugar exposure ($P = 0.03$).

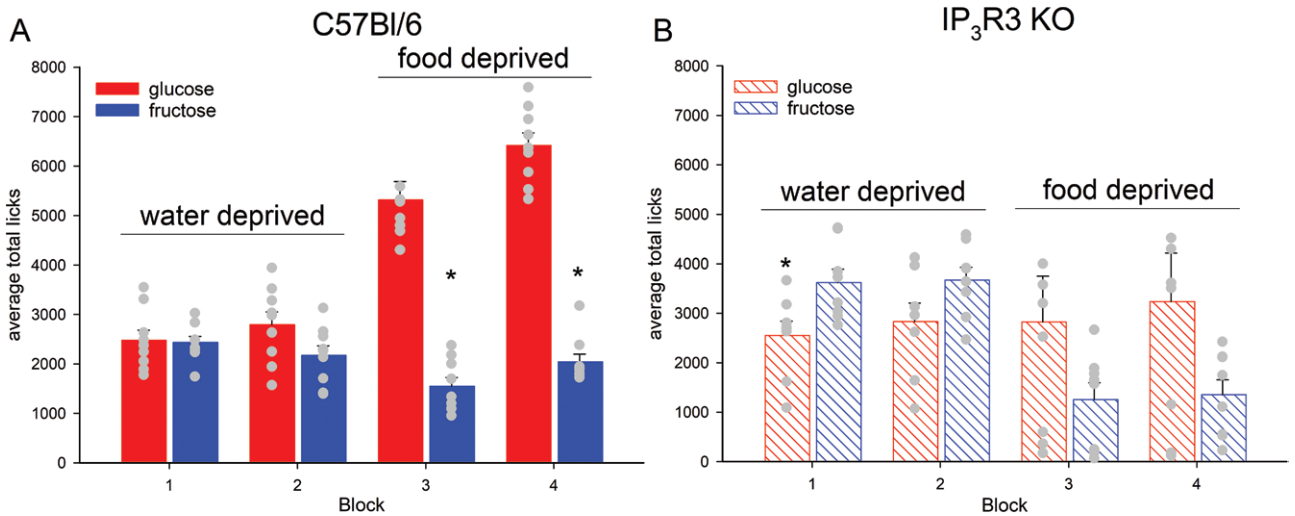

Fig. 6. (A and B) Average total licks per block for glucose and fructose collapsed across concentrations. (A, left) B6 WT mice do not differ in their intake for glucose and fructose in blocks 1 and 2 while water deprived; however, they show a significantly higher intake for glucose over fructose in blocks 3 and 4 while food deprived (*P*s < 0.001). (B, right) IP_3R3 KO mice licked more for fructose over glucose in block 1 ($P < 0.01$) but not block 2 while water deprived and show no difference in intake between glucose and fructose in blocks 3 and 4. * *P*s < 0.05.

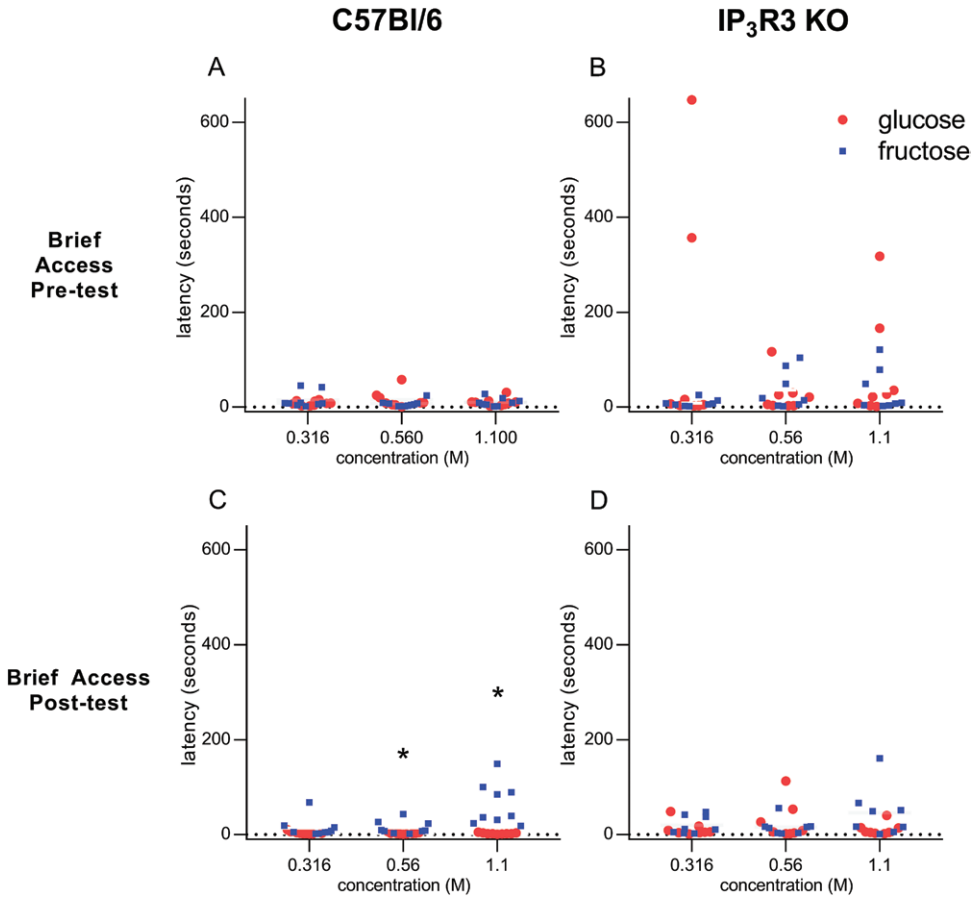

Fig. 7. (A–D) Latency to initiate licking in brief-access trials for glucose and fructose in B6 WT and IP_3R3 KO mice before and after sugar exposure. Trial latencies for glucose (red circle) and fructose (blue square) at the 3 test concentrations for each individual C57Bl/6 and IP_3R3 KO mouse before (pretest) and after (posttest) single-access sugar exposure. (A, top left) Prior to sugar exposure, B6 WT mice do not differ in their latencies to initiate licking for glucose and fructose trials at any concentration. (B, top right) Prior to sugar exposure, IP_3R3 KO mice did not differentiate in their latencies to initiate glucose and fructose trials. (C, bottom left) After sugar exposure, B6 WT mice took longer to initiate licking on fructose than glucose at the 0.56 and 1.1 M concentrations ($P = 0.035$ and $P = 0.011$, respectively). (D, bottom right) After sugar exposure, IP_3R3 KO mice still displayed statistically similar latencies to initiate glucose and fructose trials at each concentration. * Ps < 0.05.

sensing and can contribute to understanding the reinforcing nature of consuming sugars and carbohydrates.

Acknowledgments

We would like to thank Grace Kim, Miranda Berkebile, and Kyle Zumpano for technical assistance.

Author contributions

Experiments were designed by A-MT, KFM, and LAS. Data were collected by VAG, LEM, and KFJ. Data were analyzed by VAG, LEM, ASR, LAS, and A-MT. Manuscript was prepared by A-MT, LAS, VAG, LEM, and KFM.

Funding

This work was supported by National Science Foundation (grant number IOS 1942291 [to A-MT], grant number IOS 1949989 [to KFM]) and National Institutes of Health (grant number R01DC018562 [to LAS], grant number DC016869 [to A-MT]) and by the National Institutes of Health, National Institute of General Medical Science Awards (R25 GM095456 and T32 GM144920) to University at Buffalo (VA-G Trainee).

Conflict of interest

None declared.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

Andrewson TS, Martin LE, Lim J, Penner MH. Chromatographic fractionation of food-grade oligosaccharides: recognizing and avoiding sensory-relevant impurities. Food Chem. 2023:401:134071. https://doi.org/10.1016/j.foodchem.2022.134071

Ascencio Gutierrez V, Simental Ramos A, Khayoyan S, Schier LA. Dietary experience with glucose and fructose fosters heightened avidity for glucose-containing sugars independent of TRPM5 taste transduction in mice. Nutr Neurosci. 2022;26(4):1–12. https://doi.org/10.1080/1028415X.2022.2050092

Bachmanov AA, Tordoff MG, Beauchamp GK. Sweetener preference of C57BL/6ByJ and 129P3/J mice. Chem Senses. 2001:26(7):905–913. https://doi.org/10.1093/chemse/26.7.905

Chometton S, Jung AH, Mai L, Dal Bon T, Ramirez AO, Pittman DW, Schier LA. A glucokinase-linked sensor in the taste system

contributes to glucose appetite. Mol Metab. 2022:64:101554. https://doi.org/10.1016/j.molmet.2022.101554
Dutta Banik D, Benfey ED, Martin LE, Kay KE, Loney GC, Nelson AR, Ahart ZC, Kemp BT, Kemp BR, Torregrossa A-M, et al. A subset of broadly responsive Type III taste cells contribute to the detection of bitter, sweet and umami stimuli. PLoS Genet. 2020:16(8):e1008925. https://doi.org/10.1371/journal.pgen.1008925
Dutta Banik D, Martin LE, Freichel M, Torregrossa A-M, Medler KF. TRPM4 and TRPM5 are both required for normal signaling in taste receptor cells. Proc Natl Acad Sci USA. 2018:115(4):E772–E781. https://doi.org/10.1073/pnas.1718802115
Dutta Banik D, Medler KF. Defining the role of TRPM4 in broadly responsive taste receptor cells. Front Cell Neurosci. 2023:17:1148995. https://doi.org/10.3389/fncel.2023.1148995
Elizalde G, Sclafani A. Starch-based conditioned flavor preferences in rats: influence of taste, calories and CS-US delay. Appetite. 1988:11(3):179–200. https://doi.org/10.1016/s0195-6663(88)80002-3
Giza BK, Scott TR, Sclafani A, Antonucci RF. Polysaccharides as taste stimuli: their effect in the nucleus tractus solitarius of the rat. Brain Res. 1991:555(1):1–9. https://doi.org/10.1016/0006-8993(91)90852-m
Glendinning JI, Frim YG, Hochman A, Lubitz GS, Basile AJ, Sclafani A. Glucose elicits cephalic-phase insulin release in mice by activating K(ATP) channels in taste cells. Am J Physiol Regul Integr Comp Physiol. 2017:312(4):R597–R610. https://doi.org/10.1152/ajpregu.00433.2016
Glendinning JI, Maleh J, Ortiz G, Touzani K, Sclafani A. Olfaction contributes to the learned avidity for glucose relative to fructose in mice. Am J Physiol Regul Integr Comp Physiol. 2020:318(5):R901–R916. https://doi.org/10.1152/ajpregu.00340.2019
Glendinning JI, Stano S, Holter M, Azenkot T, Goldman O, Margolskee RF, Vasselli JR, Sclafani A. Sugar-induced cephalic-phase insulin release is mediated by a T1r2+T1r3-independent taste transduction pathway in mice. Am J Physiol Regul Integr Comp Physiol. 2015:309(5):R552–R560. https://doi.org/10.1152/ajpregu.00056.2015
Han SK, Mancino V, Simon MI. Phospholipase Cbeta 3 mediates the scratching response activated by the histamine H1 receptor on C-fiber nociceptive neurons. Neuron. 2006:52(4):691–703. https://doi.org/10.1016/j.neuron.2006.09.036
Hegg CC, Jia C, Chick WS, Restrepo D, Hansen A. Microvillous cells expressing IP3 receptor type 3 in the olfactory epithelium of mice. Eur J Neurosci. 2010:32(10):1632–1645. https://doi.org/10.1111/j.1460-9568.2010.07449.x
Lapis TJ, Penner MH, Lim J. Evidence that humans can taste glucose polymers. Chem Senses. 2014:39(9):737–747. https://doi.org/10.1093/chemse/bju031
Lapis TJ, Penner MH, Lim J. Humans can taste glucose oligomers independent of the hT1R2/hT1R3 sweet taste receptor. Chem Senses. 2016:41(9):755–762. https://doi.org/10.1093/chemse/bjw088
Liman ER. TRPM5 and taste transduction. Handb Exp Pharmacol. 2007;179:287–298. https://doi.org/10.1007/978-3-540-34891-7_17
Martin LE, Nikonova LV, Kay K, Paedae AB, Contreras RJ, Torregrossa AM. Salivary proteins alter taste-guided behaviors and taste nerve signaling in rat. Physiol Behav. 2018:184:150–161. https://doi.org/10.1016/j.physbeh.2017.11.021
Pullicin AJ, Penner MH, Lim J. Human taste detection of glucose oligomers with low degree of polymerization. PLoS One. 2017:12(8):e0183008. https://doi.org/10.1371/journal.pone.0183008
Ramirez I. Does starch taste like polycose®? Physiol Behav. 1991:50(2):389–392. https://doi.org/10.1016/0031-9384(91)90083-z
Schier LA, Inui-Yamamoto C, Blonde GD, Spector AC. T1R2+T1R3-independent chemosensory inputs contributing to behavioral discrimination of sugars in mice. Am J Physiol Regul Integr Comp Physiol. 2019:316(5):R448–R462. https://doi.org/10.1152/ajpregu.00255.2018
Schier LA, Spector AC. Behavioral evidence for more than one taste signaling pathway for sugars in rats. J Neurosci. 2016:36(1):113–124. https://doi.org/10.1523/JNEUROSCI.3356-15.2016
Sclafani A. Carbohydrate taste, appetite, and obesity: an overview. Neurosci Biobehav Rev. 1987:11(2):131–153. https://doi.org/10.1016/S0149-7634(87)80019-2
Sclafani A. Carbohydrate appetite in rats: taste and postingestive factors. Appetite. 1988:11:20–25. https://doi.org/10.1016/S0195-6663(88)80042-4
Sclafani A, Ackroff K. Flavor preference conditioning by different sugars in sweet ageusic Trpm5 knockout mice. Physiol Behav. 2015:140:156–163. https://doi.org/10.1016/j.physbeh.2014.12.027
Sclafani A, Nissenbaum JW, Vigorito M. Starch preference in rats. Neurosci Biobehav Rev. 1987:11(2):253–262. https://doi.org/10.1016/s0149-7634(87)80033-7
Sclafani A, Zukerman S, Ackroff K. Residual glucose taste in T1R3 knockout but not TRPM5 knockout mice. Physiol Behav. 2020:222:112945.https://doi.org/10.1016/j.physbeh.2020.112945
Smith J. The history of the "Davis Rig". Appetite. 2001:36(1):93–98. https://doi.org/10.1006/appe.2000.0372
Smith KR, Spector AC. Detection of maltodextrin and its discrimination from sucrose are independent of the T1R2 + T1R3 heterodimer. Am J Physiol Regul Integr Comp Physiol. 2017:313(4):R450–R462. https://doi.org/10.1152/ajpregu.00049.2017
Sukumaran SK, Yee KK, Iwata S, Kotha R, Quezada-Calvillo R, Nichols BL, Mohan S, Pinto BM, Shigemura N, Ninomiya Y, et al. Taste cell-expressed α-glucosidase enzymes contribute to gustatory responses to disaccharides. Proc Natl Acad Sci USA. 2016:113(21):6035–6040. https://doi.org/10.1073/pnas.1520843113
Torregrossa AM, Nikonova L, Bales MB, Villalobos Leal M, Smith JC, Contreras RJ, Eckel LA. Induction of salivary proteins modifies measures of both orosensory and postingestive feedback during exposure to a tannic acid diet. PLoS One. 2014:9(8):e105232. https://doi.org/10.1371/journal.pone.0105232
Treesukosol Y, Blonde GD, Spector AC. T1R2 and T1R3 subunits are individually unnecessary for normal affective licking responses to Polycose: implications for saccharide taste receptors in mice. Am J Physiol Regul Integr Comp Physiol. 2009:296(4):R855–R865. https://doi.org/10.1152/ajpregu.90869.2008
Treesukosol Y, Spector AC. Orosensory detection of sucrose, maltose, and glucose is severely impaired in mice lacking T1R2 or T1R3, but Polycose sensitivity remains relatively normal. Am J Physiol Regul Integr Comp Physiol. 2012:303(2):R218–R235. https://doi.org/10.1152/ajpregu.00089.2012
Woolnough JW, Bird AR, Monro JA, Brennan CS. The effect of a brief salivary α-amylase exposure during chewing on subsequent in vitro starch digestion curve profiles. Int J Mol Sci. 2010:11(8):2780–2790. https://doi.org/10.3390/ijms11082780
Xie W, Samoriski GM, McLaughlin JP, Romoser VA, Smrcka A, Hinkle PM, Bidlack JM, Gross RA, Jiang H, Wu D. Genetic alteration of phospholipase C beta3 expression modulates behavioral and cellular responses to mu opioids. Proc Natl Acad Sci U S A. 1999:96(18):10385–10390. https://doi.org/10.1073/pnas.96.18.10385
Yasumatsu K, Ohkuri T, Yoshida R, Iwata S, Margolskee RF, Ninomiya Y. Sodium-glucose cotransporter 1 as a sugar taste sensor in mouse tongue. Acta Physiol (Oxf). 2020:230(4):e13529. https://doi.org/10.1111/apha.13529
Zukerman S, Glendinning JI, Margolskee RF, Sclafani A. T1R3 taste receptor is critical for sucrose but not Polycose taste. Am J Physiol Regul Integr Comp Physiol. 2009:296(4):R866–R876. https://doi.org/10.1152/ajpregu.90870.2008
Zukerman S, Glendinning JI, Margolskee RF, Sclafani A. Impact of T1r3 and Trpm5 on carbohydrate preference and acceptance in C57BL/6 mice. Chem Senses. 2013:38(5):421–437. https://doi.org/10.1093/chemse/bjt011