Brief Communication

MOLECULAR METABOLISM

A glucokinase-linked sensor in the taste system contributes to glucose appetite

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ABSTRACT

Objectives: Dietary glucose is a robust elicitor of central reward responses and ingestion, but the key peripheral sensors triggering these orexigenic mechanisms are not entirely known. The objective of this study was to determine whether glucokinase, a phosphorylating enzyme with known glucosensory roles, is also expressed in taste bud cells and contributes to the immediate hedonic appeal of glucose-containing substances.

Methods and results: Glucokinase (*GCK*) gene transcripts were localized in murine taste bud cells with RNAScope®, and *GCK* mRNA was found to be upregulated in the circumvallate taste papillae in response to fasting and after a period of dietary access to added simple sugars in mice, as determined with real time-qPCR. Pharmacological activation of glucokinase with Compound A increased primary taste nerve and licking responses for glucose but did not impact responsivity to fructose in naïve mice. Virogenetic silencing of glucokinase in the major taste fields attenuated glucose-stimulated licking, especially in mice that also lacked sweet receptors, but did not disrupt consummatory behaviors for fructose or the low-calorie sweetener, sucralose in sugar naïve mice. Knockdown of lingual glucokinase weakened the acquired preference for glucose over fructose in sugar-experienced mice in brief access taste tests.

Conclusions: Collectively, our data establish that glucokinase contributes to glucose appetition at the very first site of nutrient detection, in the oral cavity. The findings expand our understanding of orosensory inputs underlying nutrition, metabolism, and food reward.

Keywords Sugar sensing; Nutrient assimilation; Metabolism; Food reward; Gustation; Ingestive motivation

1. INTRODUCTION

Sensory information arising from the oral cavity is critical for recognizing nutrient sources in the environment and initiating ingestive episodes. Many mammals are hardwired to seek out and preferentially consume dietary glucose [1]. Oral "sweet" taste receptors, which bind all the simple sugars, low-calorie sweeteners and some D-amino acids, play a significant role in transducing the initial sensory events that reinforce sugar consumption and give rise to their rewarding sensations [2–7], but they are not *necessary* to taste glucose, especially under certain dietary conditions. Other leadoff sensors must, therefore, subsidize rapid glucose detection [8–11]. Glucose-sensing cells exist in every major metabolic organ in the body, and many of these use glucokinase (GCK) as a detector [12–14]. Unlike other hexokinases, the rate of the catalytic steps by GCK increases as the concentration of its substrate rises, effectively allowing the cell to calibrate its output to extracellular glucose concentration [15,16]. The transcriptomes of two taste receptor cell populations—type II cells that express T1R3, linked to sweet and umami taste reception, and physiologically identified type III cells were profiled [17,18]. In a search of the supplementary database [18], we found that GCK is preferentially expressed in Type III cells. Indeed, some Type III cells are responsive to sugar [19]. However, it is unknown whether the activity of taste-bound GCK gives rise to the events that enable glucose perception and drive consumption. Considering cephalic sensory processing is critical for promoting carbohydrate intake and priming metabolism, it is important to more fully understand how the body senses glucose at this very first site of nutrient assimilation.

2. METHODS

2.1. Animals

Male C57BL6/J (B6) adult mice (between 10 and 26 weeks of age at the start of the study, n = 190) were purchased from Jackson Laboratories (Bar Harbor, ME). Male mice (~12 months old) lacking both the Tas1r2 gene and the Tas1r3 gene [T1R2+3 double knockout (KO)] on a pure C57BL6/J background (n = 10) were generously provided by Dr. Alan Spector (Florida State University, Tallahassee, FL). A TRPM5-GFP male mouse (~14 weeks old), was obtained from breeder pairs that were generously provided by Dr. Emily Liman. All mice were singly housed in standard shoebox cages with ALPHA-dri® bedding (Shepard Specialty Papers, Milford, NJ). The colony room was maintained on a 12:12 h light–dark cycle with controlled temperature and humidity. Mice had free access to standard laboratory chow (Purina #5053 or Teklad #5604 for electrophysiology experiments) and water, except when specified below. A cotton-fiber nestlet was provided in each home cage for environmental enrichment.

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All behavioral and tissue harvest procedures were approved by the *University of Southern California Institutional Animal Care and Use Committee* and all electrophysiological procedures were approved by the *Wofford College Animal Care and Use Committee* and conducted in accordance with the *National Institute of Health Guide for the Care and Use of Laboratory Animals* guidelines.

2.2. Behavioral experiments

2.2.1. Fasting conditions

To analyze whether the *GCK* mRNA expression in the circumvallate papillae (CV), the region at the back of the tongue with a high concentration of taste buds (see Figure 1M), is regulated by the energy status, B6 mice were divided into four groups. The Control mice (Fed) were maintained on a standard chow diet until the CV harvest. The Acute mice were food deprived 21–24 h before the CV harvest. The Intermittent mice (Int) were subjected to an alternation of 24 h ad libitum feeding/24 h food deprivation for one week; finishing with a 21–24 h food deprivation before the CV harvest. The Calorie Restricted mice (CR) were partially food-restricted for one week to achieve 85% of their ad libitum body weight before the CV harvest.

2.2.2. Pharmacological activation of glucokinase and short-term glucose intake

All mice were trained to drink in the gustometer (Florida State Instrumentation shop, Tallahassee, FL) in 20-minute sessions for water (6 sessions), 0.6 M glucose (2 sessions), and 0.6 M fructose (2 sessions), whilst water restricted. After training, mice were allowed to replete in the home cage and then were partially food and water restricted overnight (45 mg/kg chow; 45 ml/kg water) for the final test. In this test, the CpdA group was offered access to 0.15 M glucose solution containing 10 μM Compound A (CpdA [16], Calbiochem 603108-44-7) to drink in the gustometer for 10 min or 300 licks, whichever came first. The control group had access to 0.15 M glucose solution containing 0.03% DMSO (vehicle) instead.

2.2.3. Glucokinase knock-down (KD) and short-term sweetener intake

All mice were first trained to lick for fluid in the gustometer, and divided in two subgroups, control and GCK KD (see section 2.4). Five days after the treatment, overnight food deprived mice were subjected to the final test. In this test, the mice were offered access to 0.56 M glucose, 0.56 M fructose, or 20 mM sucralose solution to drink in the gustometer for 10 min or 300 licks, whichever came first.

2.2.4. Sugar naïve (SN) versus sugar-exposed (SE) mice

To analyze whether the *GCK* mRNA expression in the CV is regulated by the dietary status (study 1), and the effects of glucokinase stimulation (study 2) or silencing (study 3) on taste-driven licking responses to glucose versus fructose in sugar-exposed animals, B6 mice were separated in two groups within each study: sugar naïve (SN) and sugar-exposed (SE). In all studies, mice were maintained at 85% ad libitum body weight through daily chow rationing. The SE mice were subjected to single-access sugar exposure for 18 days, then all mice (SE and SN) were subjected to the glucose versus fructose (GvF) brief-access taste test (see Supplementary Fig. 1, Video S1, and [8]).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.molmet.2022.101554

In study 1, SN and SE mice were then used to analyze lingual *GCK* mRNA expression by RT-qPCR. Two days after the GvF test, taste epithelia from the CV were harvested from both groups of mice. Just prior to sacrifice, all mice were given short term access to 0.56 M glucose solution in the Davis Rig (300 licks, Dilog Instruments, Tallahassee, FL; Med Associates, Fairfax, VT).

In study 2, SN and SE mice were used to analyze the effect of lingual glucokinase stimulation on taste-based specific appetite for glucose. The two groups of mice were subjected to a final series of brief-access taste tests in the Davis Rig (each 20 min, 10-s trials, 7.5-s intertrial intervals; one test/day). Half of the mice from each group were given access to dH_2O, 0.07 M and 0.15 M glucose solutions with 10 μM CpdA or 0.03% DMSO (vehicle) added. The other half were given access to dH_2O and equimolar fructose solutions with or without CpdA added. Two days later, the test was repeated for each group with the alternate sugar.

In study 3, SN and SE mice were used to analyze the effect of lingual GCK knock-down on the ability to behaviorally discriminate glucose from fructose. Two days after the GvF test, mice were subjected to the GCK KD or control surgeries (see section 2.4 GCK knock-down'). Five days after this treatment, mice were retested for the GvF discrimination in brief access taste test.

2.3. Lick analyses

2.3.1. Licking microstructure

Mice lick in stereotypic oromotor patterns characterized by runs of consecutive licks (burst) separated by pauses (>1000 ms) [20]. A custom-written macro was used to automatically sort the time-stamped lick records to calculate total licks, burst size (number of licks per burst), total number of bursts, first burst size and cumulative burst size across the first 10 bursts.

2.3.2. Brief-access taste tests

Only mice that took at least two trials per solution were included in the analyses. The number of licks for each stimulus was averaged across trials. Then, a lick score for each stimulus was calculated as follows: lick score = mean licks to stimulus − mean licks to dH_2O.

2.4. GCK knock-down

Mice were anesthetized with isoflurane (5% induction rate; 2–3% maintenance rate, as needed) to receive either the control shRNA lentiviral particles (sc-108080, Santa Cruz Biotechnology, for the control) or the GCK shRNA (m) lentiviral particles (sc-35459-V, Santa Cruz Biotechnology, for the GCK KD) on the tongue. Briefly, 1 μl of the virus was dropped on the front part of the tongue, and 1 μl on the back part of the tongue, targeting the CV. We verified the efficiency of the GCK knock-down with this procedure using two methods. A total of 10 controls and 10 GCK KD were subjected to treatment as described above. For one subset, the CVs were harvested and the *GCK* mRNA expression was analyzed by RT-qPCR (n = 6/treatment condition, see paragraph below and Supplementary Fig. 2A). For another subset, the mice were transcardially perfused with saline followed by 4% paraformaldehyde. The excised tongues were then further postfixed with 4% paraformaldehyde solution, cryoprotected, and cut using a cryostat. GCK was labeled using RNAscope on 3–4 CVs and foliate sections per group (see section 2.8 Fluorescent In Situ Hybridization (FISH), RNAScope and Supplementary Fig. 2B).

For the sugar naïve versus sugar-exposed mice, the procedure was repeated a second time, with three hours in between treatments. Half of the mice in the control group were treated with the control virus; the other half was treated with 0.9% saline solution.

2.5. Circumvallate tissue harvest and RT-qPCR

Mice received a lethal overdose of Euthasol® (780 mg pentobarbital sodium and 100 mg phenytoin sodium per kg body weight,

intraperitoneal, IP) and then were rapidly decapitated. The whole tongue was removed and pinned into a Sylgard® dish filled with Tyrode's buffer. Under a microscope, 0.5 ml of an enzyme cocktail [1 mg/ml collagenase A (#11088793001, Sigma—Aldrich) and 2.5 mg/ml dispase II (#D4693, Sigma—Aldrich) in Tyrode's solution or phosphate-buffered saline (PBS)] was injected under the CV epithelium. Then, the tongue was placed in an oxygenated tube containing the Tyrode's solution and gently agitated with O_2 at room temperature for 20 min. After this, the CV epithelium was carefully peeled from the underlying connective tissue under the dissecting microscope. The CVs were stored overnight at 4 °C in RNAlater (ThermoFisher Scientific), then transferred in an empty tube and stored at −80 °C.

Total RNA was extracted from each sample with the RNeasy Micro Kit (Cat No. 74004, Qiagen) following the manufacturer's instructions. The total RNA concentration per sample was measured with a NanoDrop Spectrophotometer (ND-ONE-W, ThermoFisher Scientific). RNA was reverse transcribed to cDNA using the QuantiTect Reverse Transcription Kit (Cat No. 205311, Qiagen) following the manufacturer's instructions. The cDNA from the GCK KD (and control) samples were amplified using the TaqMan PreAmp Master Mix (Cat No. 4391128, Applied Biosystems). Real-time PCR was performed with TaqMan Fast Advanced Master Mix (Cat No. 4444557, Applied Biosystems) using the QuantStudio 5 Real-Time PCR System (Applied Biosystems). The following TaqMan Gene Expression Assay (ThermoFisher Scientific) were used: mouse β-actin (Actb, Mm02619580_g1), glucokinase (Gck, Mm00439129_m1), and Taste receptor type 1 member 3 (Tas1r3, Mm00473459_g1). All reactions were run in triplicate and results were normalized to β-actin expression. No template controls were included to verify the absence of genomic DNA contamination. The comparative ΔΔCt method [21] was used to quantify differences in the expression levels of our genes of interest between groups.

2.6. Electrophysiology

2.6.1. Electrophysiological recordings

Whole nerve electrophysiological recordings were obtained from the chorda tympani branch of the facial nerve innervating the taste receptor cells of the anterior, right, ipsilateral tongue. Fasted mice were anesthetized with an intraperitoneal injection of ketamine (30 mg/kg body weight) followed by urethane (1.3 g/kg body weight). Supplemental injections of urethane were administered as needed when pinching of the hind-foot elicited the flexion withdrawal reflex. Body temperature was assessed by a rectal thermometer and maintained using a homoeothermic blanket (Harvard Apparatus). Prior to taste stimulation during the surgical preparation, the tongue was kept moist with saline-soaked cotton swabs.

After a level of deep anesthesia was achieved and following tracheotomy, the mouse was placed in a non-traumatic head holder and the right-side chorda tympani nerve was exposed using a mandibular approach. The chorda tympani nerve was transected at the proximal end and desheathed. The desheathed, whole nerve was placed on a tungsten wire recording electrode and coated with Vaseline to prevent drying. A similar tungsten wire indifferent electrode was placed in the underlying muscle tissue near the base of the nerve allowing differential amplification (x 10,000, A-M Systems) of the afferent gustatory signals. The electrodes as well as the animal were grounded to the recording table. The neural responses were digitized and collected at a rate of 2,000 samples/second by a Micro1401 processor and Spike2 software (Cambridge Electronic Design).

Rinses and stimulus solutions were applied to the anterior portion of the tongue using a custom-stimulus delivery system (DiLog Instruments) which allows a continuous and constant flow rate of 100 μl per second to uphold temperature adaptation and eliminate transient tactile responses. Just prior to being presented to the tongue, the rinse and stimulus solutions flowed through a heat exchange device maintaining the stimuli at a constant temperature (35 °C) throughout the experiment. A suction tube was positioned underneath the tongue to remove excess fluid following oral stimulation. The custom stimulus delivery system was integrated with the Spike2 data collection software to allow controlled presentations of the taste stimulus solutions on command. Uninterrupted rinses with 10 μM CpdA preceded trials with 10 μM CpdA plus tastant (glucose or fructose), while 0.03% DMSO rinse solutions preceded trials with tastant in vehicle. All taste stimuli were presented for a duration of 20 s with at least 10 s of appropriate rinses after the neural signal returned to baseline. Decreasing concentrations of either fructose or glucose (0.15 M or 0.075 M) with and without CpdA were presented in randomly ordered blocks that were bracketed by stimulations of 0.5 M ammonium chloride (NH_4Cl). NH_4Cl was used as a control stimulus to assess neural responsiveness and allow normalization of the responses during the course of a recording and across subjects.

2.6.2. Data analysis

The raw nerve response was integrated using a root mean square (RMS) calculation with a time constant of 1 s. The area under the curve was measured for the first 10 s of each taste stimulus, and the 10 s immediately preceding each taste stimulus was measured as the baseline spontaneous nerve activity. The baseline measurement was subtracted from the taste stimulus measurement to produce the taste response measurement. All taste response measurements were normalized (stimulus/NH_4Cl) to the responses elicited by 0.5 M NH_4Cl stimulations bracketing the taste concentration series.

2.7. Tissue preparation for histological analyses

A B6 mouse and a TRPM5-GFP mouse were fasted ∼22 h prior to sacrifice. Then, the mice were given an intraperitoneal injection of a lethal overdose of Euthasol® (780 mg pentobarbital sodium and 100 mg phenytoin sodium per kg body weight) then perfused intracardially with 0.9% saline followed by 4% paraformaldehyde (PFA) solution. The tongue was removed and kept in 4% PFA overnight at 4 °C, then transferred to a solution containing 20% sucrose in PBS at 4 °C before being cut using a cryostat (Leica). Twenty-micron thick sections in the CV were mounted on poly-L-lysine-coated slides and stored at −20 °C.

2.8. Fluorescent In Situ Hybridization (FISH), RNAScope

CV sections from the mice tongues were post-fixed in 4% PFA, pretreated by incubating them at 37 °C in a buffer (100 mM Tris buffer and 50 mM EDTA in dH_2O, pH 8) with 0.001% Proteinase K (Sigma P2308) and incubated with 0.25% acetic anhydride in 100 mM Triethanolamine. Slides were dehydrated in an ethanol series, and air dried. For hybridization, each CV section received a probe mix (either *GCK* mRNA probe (400971-C3)/*TRPM5* mRNA probe (453251-C2), or *GCK* mRNA probe (400971-C3)/*SNAP25* mRNA probe (516471), or *GCK* mRNA probe (400971-C3) alone; all Advanced Cell Diagnostics). Sections were incubated with the probes at 40 °C for 2 h in a HybEz oven (Advanced Cell Diagnostics). Then, reagents from the RNAscope Multiplex Fluorescent Reagent Kit v2 (323100, Advanced Cell Diagnostics) were applied according to the manufacturer's instructions in order to amplify the probe signals. Finally, sections that were incubated with the two probes mixes were coverslipped with mounting medium

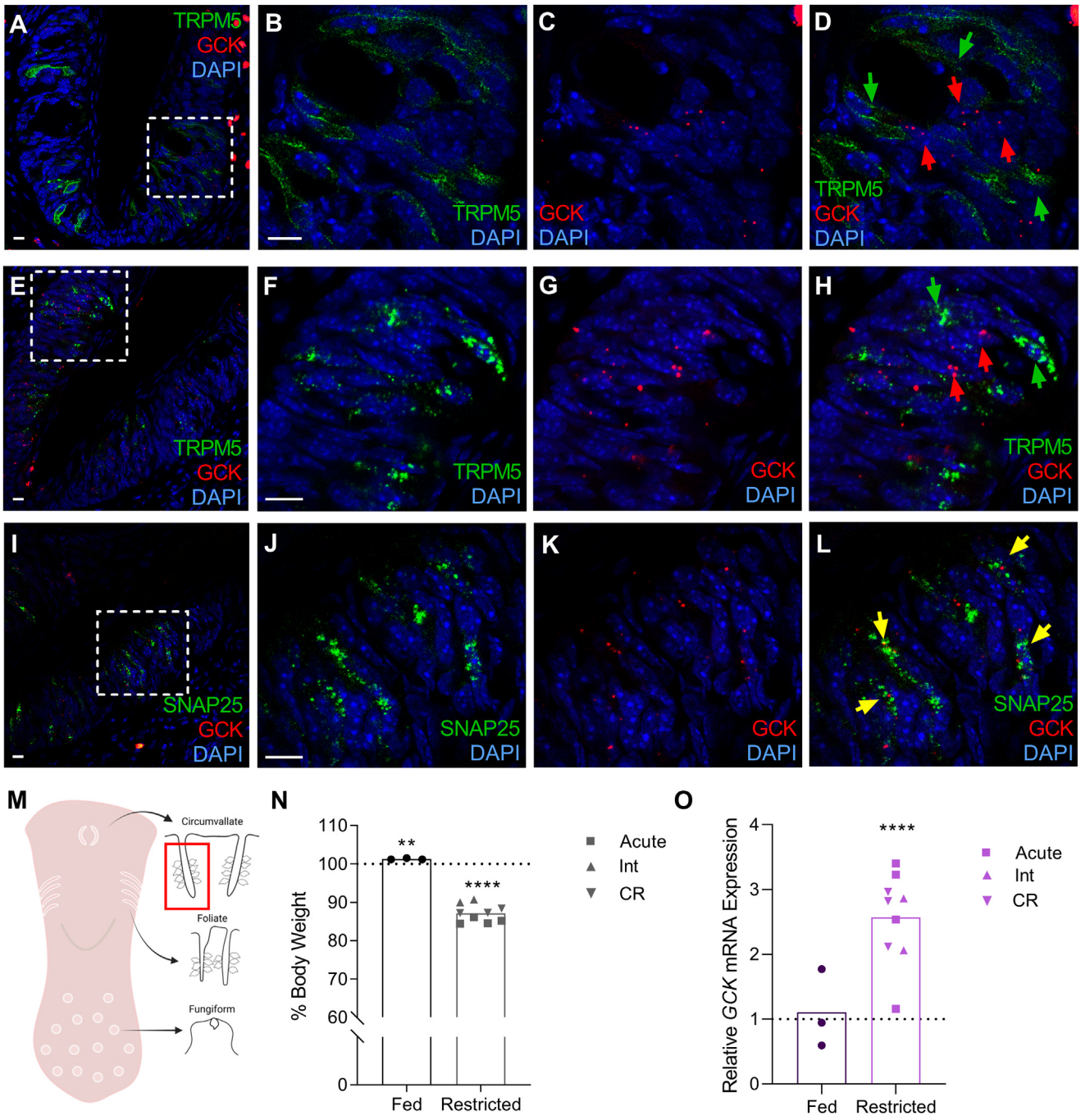

Figure 1: Glucokinase (GCK) expression in the circumvallate papillae (CV). **A.** *GCK* (red) mRNA expression was detected by FISH and TRPM5 (green) by immunohistochemistry in a TRPM5-GFP mouse CV taste buds (scale bar = 10 μm). 4′,6-diamidino-2-phenylindole (DAPI) is in blue. **B** – **D** These pictures represent a higher magnification of the white rectangle in **A** (scale bar = 10 μm): TRPM5 detection (green, **B**) and *GCK* mRNA expression (red, **C**), and the overlap (**D**) showing that the majority of *GCK* mRNA expression (red arrows) is not localized in TRPM5+ cells (green arrows). 4′,6-diamidino-2-phenylindole (DAPI) is in blue. **E.** *GCK* (red) and *TRPM5* (green) mRNA expression was detected by FISH in a B6 mouse CV taste buds (scale bar = 10 μm). 4′,6-diamidino-2-phenylindole (DAPI) is in blue. **F**–**H** These pictures represent a higher magnification of the white rectangle in **E** (scale bar = 10 μm): *TRPM5* (green, **F**) and *GCK* (red, **G**) mRNA expression, and the overlap (**H**) showing that the majority of *GCK* mRNA expression (red arrows) is not localized with *TRPM5* (green arrows). 4′,6-diamidino-2-phenylindole (DAPI) is in blue. **I.** *GCK* (red) and *SNAP25* (green) mRNA expression was detected by FISH in a B6 mouse CV taste buds (scale bar = 10 μm). 4′,6-diamidino-2-phenylindole (DAPI) is in blue. **J - L** These pictures represent a higher magnification of the white rectangle in **I** (scale bar = 10 μm): *SNAP25* (green, **J**) and *GCK* (red, **K**) mRNA expression, and the overlap (**L**) showing that the majority of *GCK* mRNA expression is localized near SNAP25 (yellow arrows). 4′,6-diamidino-2-phenylindole (DAPI) is in blue. **M.** Schematic drawing of the location of the circumvallate papillae (CV) on the back of the tongue (left), and the taste buds throughout the CV (right). The red rectangle represents the location of the pictures in A, E and I. **N.** The percentage body weight of the mice in the Fed group was higher, and that of the mice in the restricted groups was lower, than 100% (dashed line) just before the CV harvest. N = 9–12/group. The body weight of three mice is pooled per sample. Int: intermittent; CR: calorie restricted. $^{**}p < .01$; $^{****}p < .0001$. **O.** In the CV, the relative expression of *GCK* mRNA was higher than 1 (dashed line) in the restricted group, N = 9–12/group. Due to the small size of the mouse CV, we pooled 3 CVs per sample to be able to perform a real time quantitative reverse-transcription polymerase chain reaction (RT-qPCR). One Int sample was removed from the analyses because it was a statistical outlier. Int: Intermittent; CR: calorie restricted.$^{****}p < .0001$.

containing 4′,6-diamidino-2-phenylindole (DAPI) and observed under 40X objective of a confocal microscope (Zeiss LSM880) equipped with a camera. Pictures were taken using the Zeiss ZEN Lite software. Sections incubated with *GCK* mRNA probe only were then processed for immunohistochemistry.

2.9. Immunohistochemistry

CV sections from the TRPM5-GFP mouse tongue that underwent the RNAscope experiment with GCK mRNA probe were incubated with 0.3% Triton-X100 in KPBS, then with the blocking solution (2% normal donkey serum in KPBS). They were incubated with the rat TRPM5 primary antibody (#11025, BiCell Scientific) diluted in the blocking solution at 1:200 overnight at room temperature in a humidified chamber. Then, tissues were incubated with the Alexa Fluor 488 donkey anti-rat secondary antibody (A48269, Invitrogen) diluted in KPBS at 1:500 for 2 h at room temperature. Finally, sections were coverslipped with mounting medium containing 4',6-diamidino-2-phenylindole (DAPI), and observed under 40X objective of a confocal microscope (Zeiss LSM880) equipped with a camera. Pictures were taken using the Zeiss ZEN Lite software.

2.10. Statistical analyses

Statistical analyses were performed using GraphPad Prism software v8 (GraphPad Software Inc, La Jolla, CA, USA). Results are presented as mean with individual values or mean ± SEM and were considered significant with p-value $< .05$. For the fasting experiment, the electrophysiological recordings, the short-term sugar intake experiments, and the relative gene expression, Student's t-tests were used (Supplementary Table 1). For the cumulative bursts in short-term KD experiments, multiple t-tests were performed using the Benjamini, Krieger and Yekutieli FDR approach ($q = .05$; Supplementary Table 2). Finally, for the brief-access taste tests, two-way repeated-measures ANOVA were used to detect the main and interactive effects of concentration, sugar, or drug (Supplementary Table 3). *Post-hoc* comparisons between the groups were performed using Bonferroni's multiple comparison when the main and/or interactive ANOVA effects were significant.

3. RESULTS AND DISCUSSION

3.1. Glucokinase is expressed in taste bud cells

Our first goal was to confirm that glucokinase is expressed in the mouse taste bud. Double-labeling experiments revealed that GCK generally coalesces with *SNAP25*, a marker for Type III cells, and perhaps qualitatively less so with TRPM5, a marker for Type II cells in the circumvallate papillae (CV) (Figure 1A—M). GCK is also present in the foliate papillae (Supplementary Fig. 2B). The specific cell types that express GCK and their comparative distribution across the major taste fields will need to be more fully characterized in the future. Nevertheless, the results clearly corroborate [18], establishing GCK's presence in the taste buds.

3.2. Glucokinase expression in taste bud cells is increased with fasting

Glucokinase is regulated by metabolic state in other cell types [12,22]. Therefore, we next assessed whether GCK in the CV taste papillae exhibits this phenomenon. CVs were harvested from ad libitum fed mice (Fed), after a single fast (~24 h without food; Acute), after a week of intermittent fasting (24 h fed/24 h fasted cycles; Int), or a week of partial caloric restriction (to achieve 85% of their ad libitum body weight; CR). All fasting conditions led to a reduction in body mass, and, collectively drove a significant increase in GCK expression in the CV (Figure 1N—O and Supplementary Table 1). We hypothesize that metabolic programming of these primary sensory inputs may promote faster recognition of dietary glucose when immediate sources of fuel are needed.

3.3. Lingual glucokinase stimulation amplifies taste-driven glucose intake

We next assessed if glucokinase in the taste buds contributes to taste sensation and motivated behavior. First, to test the hypothesis that the activation of lingual glucokinase generates a signal that is transmitted to the brain via the peripheral gustatory system, we recorded whole nerve electrophysiological activity from the chorda tympani (CT) in response to taste stimulation with glucose or fructose solutions containing the glucokinase activator Compound A [CpdA [16]] or its vehicle in naïve B6 mice. CpdA significantly increased the taste nerve responsiveness to a perithreshold concentration of glucose, but not fructose, relative to vehicle (0.15 M; Figure 2A and Supplementary Table 1). CpdA did not affect neural responsivity to a lower, subthreshold, concentration of these sugars (0.075 M; Supplementary Fig. 3 and Supplementary Table 1).

Considering gustatory signals can drive either physiological or hedonic effectors [23], we next asked whether lingual activation stimulated taste-driven consummatory behaviors for glucose. Therefore, in a

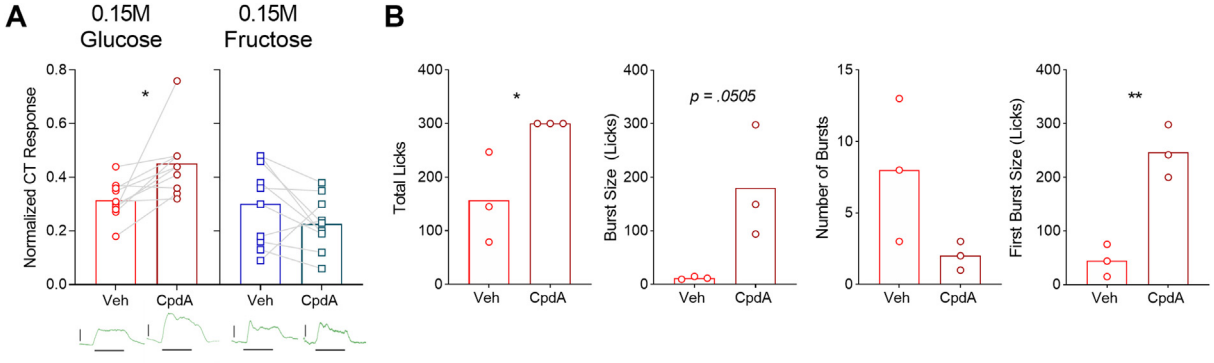

Figure 2: Lingual glucokinase stimulation amplifies taste-driven glucose intake. A. 10 μM CpdA added to 0.15 M glucose solution significantly increased the chorda tympani (CT) response compared to 0.15 M glucose added with 0.03% DMSO (vehicle) (left). The CpdA had no significant effect on equimolar fructose (right). Below each graph bar is a representative trace of the CT nerve recordings (X scale = 20 s; Y scale = 2 V). N = 10; $*p < .05$. **B.** The mean total number of licks and first burst size were increased, the average burst size was close to the significance, and the mean total number of bursts was not different, when mice had access for 10-minute (or 300 licks) to a glucose solution added with 10 μM CpdA compared to vehicle. N = 3/group; $*p < .05$; $**p < .01$.

preliminary study, a separate cohort of B6 mice was partially food and water restricted to motivate ingestion, and then offered a 0.15 M solution of glucose to lick for 300 licks (1 μl/lick) or 10 min, whichever came first. For half of the mice, CpdA was added to the solution to stimulate glucokinase activity; the other half received the same concentration of glucose with vehicle. The 300-lick cutoff was

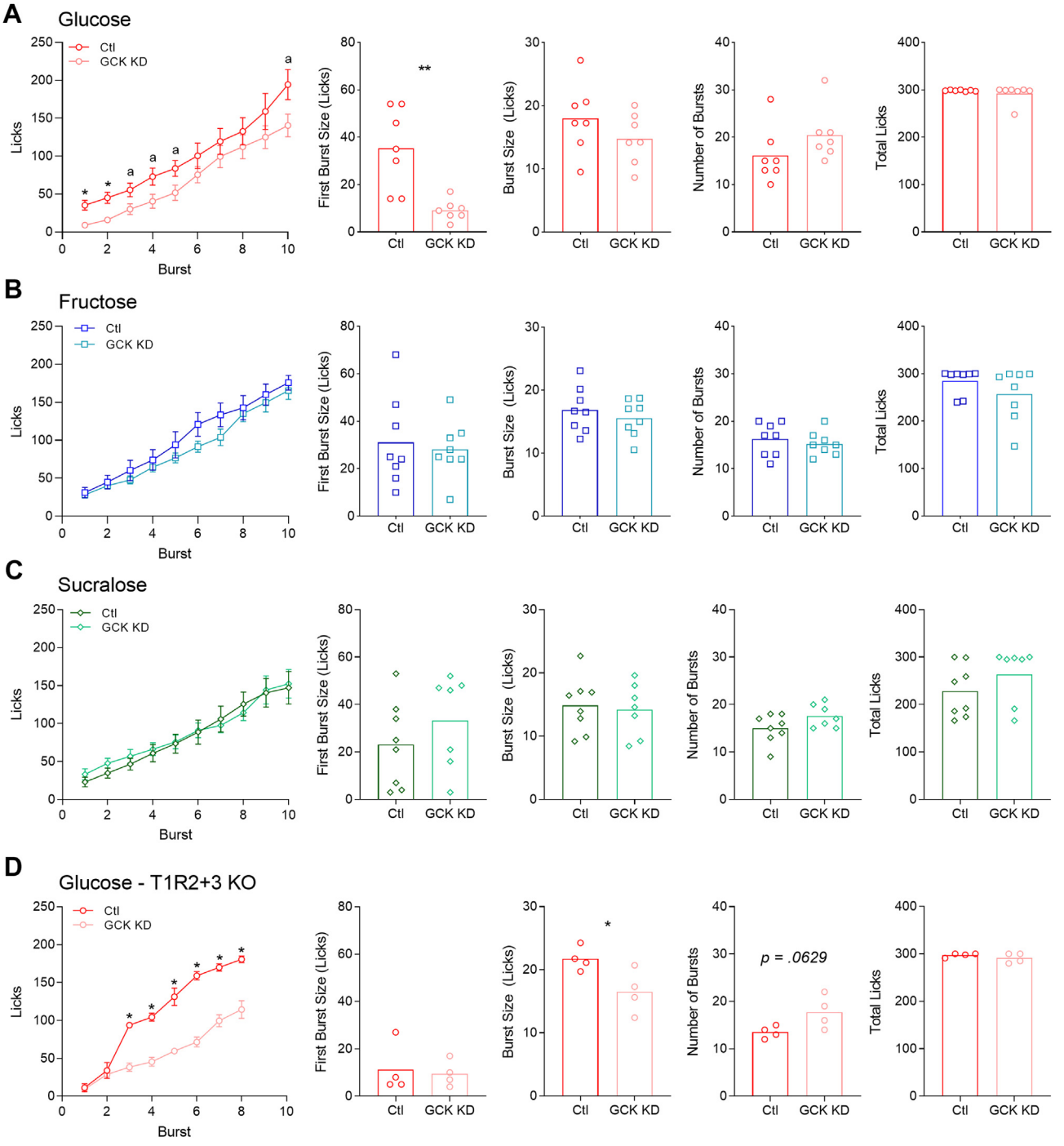

Figure 3: Lingual glucokinase silencing impairs behavioral sensitivity to glucose. **A.** The first burst size as well as the cumulative number of licks for the first five bursts was lower for the GCK knock-down (KD) mice that had access for 10-minute (or 300 licks) to a glucose solution compared to control. The average burst size, mean total number of bursts and mean total number of licks were not different. N = 7/group; $*p < .05$; $**p < .01$; *a*: $q = .072673$. **B.** The cumulative number of licks for the first ten bursts, first burst size, average burst size, mean total number of bursts or mean total number of licks were not different when GCK KD mice had access for 10-minute (or 300 licks) to a fructose solution compared to control mice. N = 8/group. **C.** The cumulative number of licks for the first ten bursts; first burst size, average burst size, mean total number of bursts or mean total number of licks were not different when GCK KD mice had access for 10-minute (or 300 licks) to a sucralose solution compared to control mice. N = 8/group. One GCK KD mice was removed from the analyses because it was a statistical outlier for one of the licking parameters. **D.** The cumulative number of licks and the average burst size were lower for the GCK KD T1R2+3 KO mice that had access for 10-minute (or 300 licks) to a glucose solution compared to control T1R2+3 KO mice. The first burst size, mean total number of bursts or mean total number of licks were not different. N = 5/group. One GCK KD and one control mouse were removed from the analyses because each was a statistical outlier for one of the licking parameters. $*p < .05$.

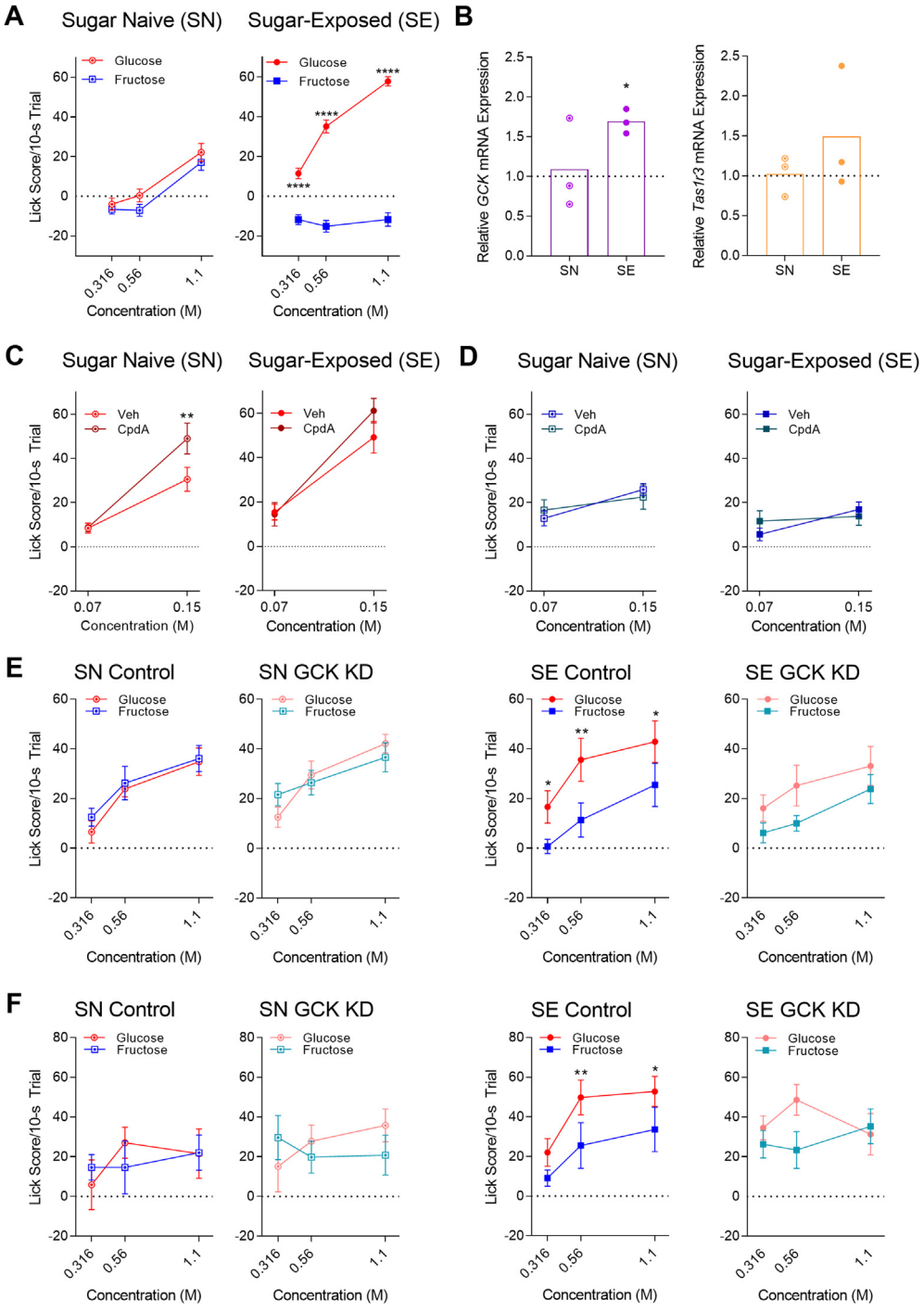

Figure 4: Lingual glucokinase is regulated by dietary sugar exposure and contributes to the experience-based enhanced avidity for glucose. A. In a glucose versus fructose (GvF) brief-access taste test, sugar-exposed mice (SE, right) licked significantly more for glucose than fructose, whereas sugar naïve mice (SN, left) licked the same for both solutions. N = 12/group; $^{****}p < .0001$. **B.** In the CV, the relative expression of *GCK* mRNA was higher than 1 (dashed line) in sugar-exposed (SE), but not in sugar naïve (SN), mice (left). The relative expression of *Tas1r3* mRNA was not different than 1 (dashed line) for both groups (right). N = 12/group. Four CVs are pooled per sample. $^{*}p < .05$.**C.** 10 μM CpdA added to 0.15 M glucose solution increased licking in a brief-access taste test for sugar naïve (SN, left), compared to equimolar solutions added with 0.03% DMSO (vehicle). 10 μM CpdA added to 0.07 M glucose solution had no effect on licking for both groups (see text for details). N = 6/group; $^{**}p < .01$. **D.** 10 μM CpdA added to fructose solutions did not modify licking in a brief-access taste test for the same sugar naïve (SN, left) and sugar-exposed (SE, right) mice. N = 6/group. **E.** In sugar naïve mice (SN), the lingual GCK knock-down (KD) surgery did not modify licking for glucose and fructose solutions in the brief-access taste test compared to SN mice after control surgery (left). In sugar-exposed mice (SE), the lingual GCK KD surgery decreased the difference in glucose versus fructose intake observed in the SE mice after control surgery (right). N = 8/group; $^{*}p < .05$; $^{**}p < .01$. **F.** When the first block of the brief-access taste test is analyzed (one trial/solution), the lingual GCK knock-down (KD) surgery in sugar naïve (SN) mice did not modify licking for glucose and fructose solutions in the brief-access taste test compared to SN mice after control surgery (left). In sugar-exposed mice (SE), the controls were able to differentiate the two solutions in the first block, while the lingual GCK KD surgery attenuated this discrimination (right). N = 8/group; $^{*}p < .05$; $^{**}p < .01$.

purposefully chosen to preclude the onset of postingestive signals during the behavioral measurement period. CpdA significantly increased the total number of licks taken on this test (Figure 2B and Supplementary Table 1). We also analyzed the microstructural organization of the licking behavior. Mice lick in a stereotypic fashion characterized by runs of temporally contiguous licks (bursts) followed by short pauses. The size of these bursts increases as sugar concentration increases (and decreases with quinine adulteration); thus, burst size is a common metric of a substance's hedonic appeal [24,25]. The microstructural analysis revealed that CpdA increased average burst size, suggesting that lingual glucokinase activation boosts the perceived hedonic value of glucose. To further confirm this was driven by taste-related input, as opposed to postingestive or metabolic influences, we analyzed the size of the first burst, which is elicited by the initial contact of the taste receptors with the glucose solution. Indeed, CpdA significantly increased first burst size (Figure 2B and Supplementary Table 1).

3.4. Lingual glucokinase silencing attenuates behavioral responsivity to glucose in sweet-sensitive and sweet-blind mice

To determine if lingual GCK is *required* to motivate licking behaviors for glucose, we used a short hairpin RNA (shRNA) packaged in a lentiviral vector to specifically target and silence GCK in the major taste fields of the oral epithelium (Supplementary Fig. 2 and Supplementary Table 1). B6 mice were treated with the GCK shRNA or a scrambled control vector. Five days later, following an overnight fast, they were given a short lick test, wherein 0.56 M glucose was offered for 300 licks (1 ul/lick) or 10 min. The 0.56 M concentration was chosen because our previous work showed that naïve food-restricted mice will lick avidly for it, allowing us to test whether loss of GCK suppresses licking [8]. Virogenetic knockdown of GCK (GCK KD) significantly reduced the number of licks elicited by glucose in the very first burst (Figure 3A and Supplementary Table 1). In this fasted state, all mice were sufficiently motivated to consume calories, and ultimately reached the automatic 300-lick cut off (Figure 3A and Supplementary Table 1), but close tracking of cumulative licking responses across the first 10 successive bursts revealed that GCK KD mice licked less vigorously for glucose in the initial bursts and failed to fully compensate for this early sensory-related deficit as the ingestive episode progressed (Figure 3A and Supplementary Table 2). To determine if the loss in taste-driven motivation as a result of GCK KD was specific to glucose, we replicated the study in two different cohorts of B6 mice, for which either a different sugar (0.56 M fructose) or low-calorie sweetener (20 mM sucralose), both ligands for the sweet receptor, were offered instead. GCK KD did not affect licking for fructose or sucralose, nor did it influence any of the microstructural licking patterns for these substances (Figure 3B–C, and Supplementary Tables 1 and 2).

Mice lacking the canonical sweet receptors still respond positively to the taste of glucose [26]. Thus, next we tested if glucokinase plays a critical role in mediating the residual attraction to glucose in sweet-*blind* mice (T1R2+3 KO) in the same 300 lick test with glucose. In the absence of the sweet receptor function, both GCK KD and control mice took small initial lick bursts (Figure 3D and Supplementary Table 1). However, whereas control T1R2+3 KO rapidly increased their licking bursts thereafter, GCK KD T1R2+3 KO mice did not. With both the sweet receptor and lingual GCK compromised, mice licked in apathetic fashion for glucose across the succeeding bursts, and essentially required more bursts to reach the 300-lick cutoff (Figure 3D, and Supplementary Tables 1 and 2).

The deficit observed following GCK knockdown in sweet-*competent* mice was most pronounced in the earliest phase of the ingestive episode with glucose. Thus, one possibility is that this glucokinase-linked sensor plays a crucial role in the initial ability to detect glucose, but other inputs (e.g., T1R2+3-dependent) are sufficient to further drive consumption as the episode continues. Consistent with this possibility, mice lacking both the T1R2+3 receptors and lingual GCK displayed a profound motivational deficit to ingest glucose, even while in a state of nutritional need. It is important to note that although the shRNA significantly reduced *GCK* expression, it did not *completely* silence *GCK*. Plus, we focused the virogenetic treatment to the two main taste fields, the fungiform and the circumvallate papillae, which left other taste fields (e.g., foliate papillae and soft palate) unaffected. For these reasons, we cannot yet exclude the possibility that residual GCK activity in other taste buds was sufficient to maintain some level of responding to glucose in both the B6 and T1R2+3 KO mice. Similarly, whether GCK generates a critical signal subserving homeostatic controls of intake remains unknown. Nevertheless, it is notable that even partial GCK knockdown in the taste system yielded reliable and significant perturbations in immediate taste-guided behaviors for glucose, leading us to the conclusion that glucokinase is an important sensory input component underlying rapid glucose detection.

3.5. Lingual glucokinase is regulated by dietary sugar exposure and contributes to the experience-based enhanced avidity for glucose

Schier et al. previously showed that rodents acquire a preference for the taste of glucose over fructose after a period of dietary experience with the two sugars, a phenomenon that does not depend on functional sweet receptors [8]. Here, we tested if gustatory glucokinase enables this hedonic sugar discrimination. First, we found that GCK was significantly elevated in the taste bud cells of sugar-exposed mice (Figure 4B, Supplementary Table 1) and this corresponded to their heightened avidity for glucose in brief access taste tests (Figure 4A, Supplementary Table 3, Supplementary Fig. 1 and Video 1). In a separate experiment, CpdA significantly enhanced licking for a peri-threshold glucose concentration in sugar naïve B6 mice (0.15 M, Figure 4C and Supplementary Table 3). While CpdA also tended to increase licking for 0.15 M glucose in sugar-exposed B6 mice, this effect did not quite reach statistical significance. We hypothesize that the endogenously high levels of GCK in the taste buds of the sugar-exposed mice (e.g., Figure 4B) were sufficient to drive licking levels to the ceiling, even in the vehicle condition, such that any additional benefit of CpdA was not observable (Figure 4C and Supplementary Table 3). Licking levels were submaximal for the 0.07 M glucose solution in both groups, and CpdA did not further enhance responsiveness to this stimulus (Figure 4C and Supplementary Table 3). This latter finding, coupled with our electrophysiological data (see Supplementary Fig. 3), hint that glucokinase may only be engaged above a certain local threshold concentration, as is the case in other cell types [27,28]. Importantly though, CpdA did not increase licking responses to water during the glucose test (data not shown), nor did it modify the responses to fructose at any concentration tested in the sugar naïve or sugar-exposed mice (Figure 4D and Supplementary Table 3).

We next tested whether glucokinase is an essential part of the sensory cascade that enables sugar-exposed mice to rapidly distinguish glucose from fructose based on taste input. After the period of dual sugar access (Supplementary Fig. 1A), subsets of mice underwent GCK KD or the control treatment. Sugar naïve mice licked comparably for both sugars in the brief access taste test (Figure 4E, Supplementary Fig. 1B and Supplementary Table 3). Notably, this profile was not affected by the KD. This testing paradigm, with rapid successive 10-s

exposures to a truncated range of suprathreshold concentrations of both sugars without washouts, may foster some stimulus cross-interference across trials and may, therefore, not be particularly sensitive to unconditioned differences in relative sugar preference amongst naïve subjects. The precise contributions of gustatory GCK to inherent taste preference and perception will need to be characterized in follow up studies with other types of psychophysical tests. Rather, the point here was to assess if lingual GCK contributed to the acquired preference for glucose over fructose. Indeed, we found that sugar-exposed mice that received the control treatment displayed the expected increase in lick responses to glucose over fructose, in this brief access taste test (Figure 4E and Supplementary Table 3). Sugar-exposed mice that underwent GCK KD also generally preferred glucose, but the magnitude of this preference was substantially diminished (Figure 4E and Supplementary Table 3). In fact, close inspection of how the mice responded to the various sugars on the first trial block (the very first contact with each solution) showed that whereas the sugar-exposed control mice readily licked more for glucose compared to fructose on these initial trials, the sugar-exposed GCK KD mice failed to discern among the two sugars (Figure 4F and Supplementary Table 3). Taken together then, the results show that GCK contributes a key sensory signal that biases immediate ingestive actions towards glucose over fructose, when the respective metabolic outcomes are known.

Dietary glucose is a prominent reinforcer of energy intake and preference [29,30]. Although glucose rouses these motivated behaviors via its activation of the oral sweet receptors, this canonical source of input alone is not enough to sustain the strong appetite for glucose-containing foods and fluids [31–33]. The present findings reveal a glucose-specific sensor in the taste system whose signal subsidizes the hedonic value of glucose, rapidly reinforces ingestion, and facilitates the discrimination of glucose from other sweet-tasting substances in the food environment, perhaps especially in times of energy demand and with nutritional experience. These findings open up new research directions aimed at understanding how gustatory glucokinase cooperates with other receptors and signaling pathways in the taste end organ in the service of nutrient assimilation, including which glucokinase isoform taste cells utilize and its subcellular localization [34–37]. Moreover, the findings underscore the role of dietary factors in tuning the primary chemosensory information coming in to key homeostatic and hedonic effectors of energy balance. The fact that added simple sugar to the diet increased GCK levels in the taste cells and led to a concomitant increase in appetite for the taste of glucose could have been advantageous in a time when food availability was more limited. However, in the modern food environment, this programmed response is now likely detrimental to health, and could be one of the factors leading to excess sugar consumption, weight gain, and diabetes. More studies are needed to unravel how physiological states, diets, and their interactions regulate GCK activity in the taste cells in health and disease.

AUTHOR CONTRIBUTIONS

SC: Data curation, formal analysis, investigation, methodology, writing-original draft; AJ: Investigation; LM: Formal analysis, investigation; TD: Formal analysis, investigation; AOR: Investigation; DWP: Formal analysis, investigation, methodology; LAS: Conceptualization, data curation, formal analysis, funding acquisition, methodology, supervision, writing-original draft.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2022.101554.

REFERENCES

[1] Remesar, X., Alemany, M., 2020. Dietary energy partition: the central role of glucose. International Journal of Molecular Sciences 21.
[2] Nelson, G., Hoon, M.A., Chandrashekar, J., Zhang, Y., Ryba, N.J., Zuker, C.S., 2001. Mammalian sweet taste receptors. Cell 106:381–390.
[3] Montmayeur, J.P., Liberles, S.D., Matsunami, H., Buck, L.B., 2001. A candidate taste receptor gene near a sweet taste locus. Nature Neuroscience 4:492–498.
[4] Kitagawa, M., Kusakabe, Y., Miura, H., Ninomiya, Y., Hino, A., 2001. Molecular genetic identification of a candidate receptor gene for sweet taste. Biochemical and Biophysical Research Communications 283:236–242.
[5] Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M., Adler, E., 2002. Human receptors for sweet and umami taste. Proceedings of the National Academy of Sciences of the United States of America 99:4692–4696.
[6] Treesukosol, Y., Spector, A.C., 2012. Orosensory detection of sucrose, maltose, and glucose is severely impaired in mice lacking T1R2 or T1R3, but Polycose sensitivity remains relatively normal. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 303:R218–R235.
[7] Hoon, M.A., Adler, E., Lindemeier, J., Battey, J.F., Ryba, N.J., Zuker, C.S., 1999. Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. Cell 96:541–551.
[8] Schier, L.A., Inui-Yamamoto, C., Blonde, G.D., Spector, A.C., 2019. T1R2+T1R3-independent chemosensory inputs contributing to behavioral discrimination of sugars in mice. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 316:R448–R462.
[9] Damak, S., Rong, M., Yasumatsu, K., Kokrashvili, Z., Varadarajan, V., Zou, S., et al., 2003. Detection of sweet and umami taste in the absence of taste receptor T1r3. Science 301:850–853.
[10] Treesukosol, Y., Smith, K.R., Spector, A.C., 2011. Behavioral evidence for a glucose polymer taste receptor that is independent of the T1R2+3 heterodimer in a mouse model. Journal of Neuroscience 31:13527–13534.
[11] Yasumatsu, K., Ohkuri, T., Yoshida, R., Iwata, S., Margolskee, R.F., Ninomiya, Y., 2020. Sodium-glucose cotransporter 1 as a sugar taste sensor in mouse tongue. Acta Physiologica 230:e13529.
[12] Hussain, S., Richardson, E., Ma, Y., Holton, C., De Backer, I., Buckley, N., et al., 2015. Glucokinase activity in the arcuate nucleus regulates glucose intake. Journal of Clinical Investigation 125:337–349.
[13] Dunn-Meynell, A.A., Routh, V.H., Kang, L., Gaspers, L., Levin, B.E., 2002. Glucokinase is the likely mediator of glucosensing in both glucose-excited and glucose-inhibited central neurons. Diabetes 51:2056–2065.
[14] Matschinsky, F., Liang, Y., Kesavan, P., Wang, L., Froguel, P., Velho, G., et al., 1993. Glucokinase as pancreatic beta cell glucose sensor and diabetes gene. Journal of Clinical Investigation 92:2092–2098.

[15] Iynedjian, P.B., 2009. Molecular physiology of mammalian glucokinase. Cellular and Molecular Life Sciences 66:27–42.
[16] Bourbonais, F.J., Chen, J., Huang, C., Zhang, Y., Pfefferkorn, J.A., Landro, J.A., 2012. Modulation of glucokinase by glucose, small-molecule activator and glucokinase regulatory protein: steady-state kinetic and cell-based analysis. Biochemical Journal 441:881–887.
[17] Roper, S.D., Chaudhari, N., 2017. Taste buds: cells, signals and synapses. Nature Reviews Neuroscience 18:485–497.
[18] Sukumaran, S.K., Lewandowski, B.C., Qin, Y., Kotha, R., Bachmanov, A.A., Margolskee, R.F., 2017. Whole transcriptome profiling of taste bud cells. Scientific Reports 7:7595.
[19] Dutta Banik, D., Benfey, E.D., Martin, L.E., Kay, K.E., Loney, G.C., Nelson, A.R., et al., 2020. A subset of broadly responsive Type III taste cells contribute to the detection of bitter, sweet and umami stimuli. PLoS Genetics 16:e1008925.
[20] Johnson, A.W., Sherwood, A., Smith, D.R., Wosiski-Kuhn, M., Gallagher, M., Holland, P.C., 2010. An analysis of licking microstructure in three strains of mice. Appetite 54:320–330.
[21] Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408.
[22] Toyoda, Y., Miwa, I., Kamiya, M., Ogiso, S., Nonogaki, T., Aoki, S., Okuda, J., 1995. Tissue and subcellular distribution of glucokinase in rat liver and their changes during fasting-refeeding. Histochemistry and Cell Biology 103(1):31–38. https://doi.org/10.1007/BF01464473.
[23] Spector, A.C., Schier, L.A., 2018. Behavioral evidence that select carbohydrate stimuli activate T1R-independent receptor mechanisms. Appetite 122:26–31.
[24] Davis, J.D., Perez, M.C., 1993. Food deprivation- and palatability-induced microstructural changes in ingestive behavior. American Journal of Physiology 264:R97–R103.
[25] Spector, A.C., Klumpp, P.A., Kaplan, J.M., 1998. Analytical issues in the evaluation of food deprivation and sucrose concentration effects on the microstructure of licking behavior in the rat. Behavioral Neuroscience 112:678–694.
[26] Sclafani, A., Zukerman, S., Ackroff, K., 2020. Residual glucose taste in T1R3 knockout but not TRPM5 knockout mice. Physiology & Behavior 222:112945.
[27] Roberts, B.L., Zhu, M., Zhao, H., Dillon, C., Appleyard, S.M., 2017. High glucose increases action potential firing of catecholamine neurons in the nucleus of the solitary tract by increasing spontaneous glutamate inputs. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 313:R229–R239.
[28] Sweet, I.R., Matschinsky, F.M., 1995. Mathematical model of beta-cell glucose metabolism and insulin release. I. Glucokinase as glucosensor hypothesis. American Journal of Physiology 268:E775–E788.
[29] Sclafani, A., Ackroff, K., 2016. Operant licking for intragastric sugar infusions: differential reinforcing actions of glucose, sucrose and fructose in mice. Physiology & Behavior 153:115–124.
[30] Sclafani, A., Zukerman, S., Ackroff, K., 2015. Postoral glucose sensing, not caloric content, determines sugar reward in C57BL/6J mice. Chemical Senses 40:245–258.
[31] de Araujo, I.E., 2016. Circuit organization of sugar reinforcement. Physiology & Behavior 164:473–477.
[32] Tellez, L.A., Han, W., Zhang, X., Ferreira, T.L., Perez, I.O., Shammah-Lagnado, S.J., et al., 2016. Separate circuitries encode the hedonic and nutritional values of sugar. Nature Neuroscience 19:465–470.
[33] de Araujo, I.E., Oliveira-Maia, A.J., Sotnikova, T.D., Gainetdinov, R.R., Caron, M.G., Nicolelis, M.A., et al., 2008. Food reward in the absence of taste receptor signaling. Neuron 57:930–941.
[34] Glendinning, J.I., Frim, Y.G., Hochman, A., Lubitz, G.S., Basile, A.J., Sclafani, A., 2017. Glucose elicits cephalic-phase insulin release in mice by activating K(ATP) channels in taste cells. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 312:R597–R610.
[35] Breslin, P.A.S., Izumi, A., Tharp, A., Ohkuri, T., Yokoo, Y., Flammer, L.J., et al., 2021. Evidence that human oral glucose detection involves a sweet taste pathway and a glucose transporter pathway. PLoS One 16:e0256989.
[36] Magnuson, M.A., 1992. Tissue-specific regulation of glucokinase gene expression. Journal of Cellular Biochemistry 48:115–121.
[37] Iynedjian, P.B., 1993. Mammalian glucokinase and its gene. Biochemical Journal 293(Pt 1):1–13.

