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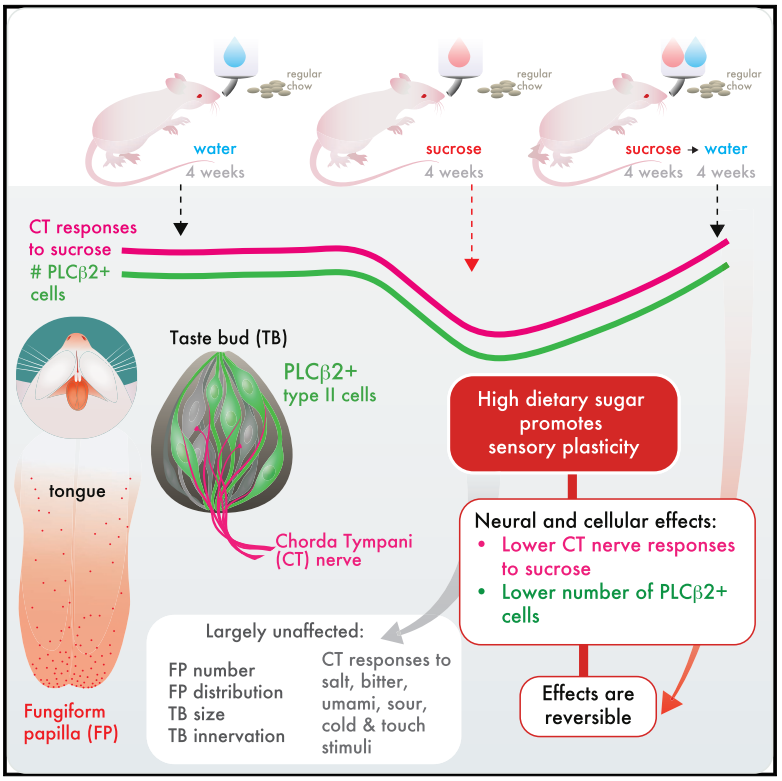
High-sucrose diet exposure is associated with selective and reversible alterations in the rat peripheral taste system

Graphical abstract



Highlights

- Sucrose consumption showed specific and selective effects on the peripheral taste system
- Dietary sugar lowered chorda tympany responses to sucrose and the number of PLCβ2+ cells
- Responses to other sensory qualities and modalities were largely unaffected
- We observed that phenotypes were restored after sucrose was removed from the diet

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In brief

Sung et al. show that elevated dietary sucrose decreases the responses of the chorda tympany nerve to sucrose. The morphology of the fungiform papilla and the number, size, and innervation of the taste buds were unaffected, but the number of PLCβ2+ cells was lower. These selective effects were restored when sucrose was removed from the diet.

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High-sucrose diet exposure is associated with selective and reversible alterations in the rat peripheral taste system

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SUMMARY

Elevated sugar consumption is associated with an increased risk for metabolic diseases. Whereas evidence from humans, rodents, and insects suggests that dietary sucrose modifies sweet taste sensation, understanding of peripheral nerve or taste bud alterations is sparse. To address this, male rats were given access to 30% liquid sucrose for 4 weeks (sucrose rats). Neurophysiological responses of the chorda tympani (CT) nerve to lingual stimulation with sugars, other taste qualities, touch, and cold were then compared with controls (access to water only). Morphological and immunohistochemical analyses of fungiform papillae and taste buds were also conducted. Sucrose rats had substantially decreased CT responses to 0.15–2.0 M sucrose compared with controls. In contrast, effects were not observed for glucose, fructose, maltose, Na saccharin, NaCl, organic acid, or umami, touch, or cold stimuli. Whereas taste bud number, size, and innervation volume were unaffected, the number of PLCβ2+ taste bud cells in the fungiform papilla was reduced in sucrose rats. Notably, the replacement of sucrose with water resulted in a complete recovery of all phenotypes over 4 weeks. The work reveals the selective and modality-specific effects of sucrose consumption on peripheral taste nerve responses and taste bud cells, with implications for nutrition and metabolic disease risk.

INTRODUCTION

Sweet-tasting foods are strongly preferred by people and many other animals, despite the fact that sugars are not an essential food source.[1,2] This proclivity toward sugars has led to a range of health concerns across the ages, from increases in dental carries with the growing popularity of sucrose in Europe in the 1700s[1,3] to the higher incidence of cardiovascular and metabolic diseases more recently.[4] Although much attention has been paid to the effects of sugar consumption on ingestive behavior and metabolism,[4,5] relatively few studies have examined effects on the peripheral taste system despite the important role of taste in nutrition.[6–8]

Taste is particularly important for the selection and ingestion of carbohydrate-containing foods in humans,[9] rats,[10] and flies.[11] Interestingly, the gustatory system is sensitive to modulation by a range of environmental factors,[8,12] including disease,[13,14] drug treatment,[15] and diet.[16–19] Some data suggest that prolonged dietary sugar exposure reduces sensory responses to sucrose in flies[20–23] and female rats,[6] but several questions remain open. First, it is unclear whether sucrose exposure affects peripheral nerve responses to sucrose at both low and high concentrations. Second, little is known about the effects of high sucrose on peripheral nerve responses to other sweet stimuli as well as other taste qualities or sensory modalities such as touch and temperature. Third, the effects of high sucrose intake on the lingual taste papilla, taste buds (TBs), and taste cells remain largely uncharacterized. Finally, whether the effects of high sucrose consumption on the mammalian taste system are reversible or persistent is unknown.

To answer these questions, we gave male rats free access to liquid sucrose for 4 weeks and examined the responses of the sensory chorda tympani (CT) nerve—which innervates taste papillae on the front of the tongue—to different taste qualities and modalities and characterized changes in the anatomical structures that support taste physiology, including the fungiform papilla (FP) and the resident TB. We then assessed the recovery of sucrose-induced neurophysiological and anatomical effects

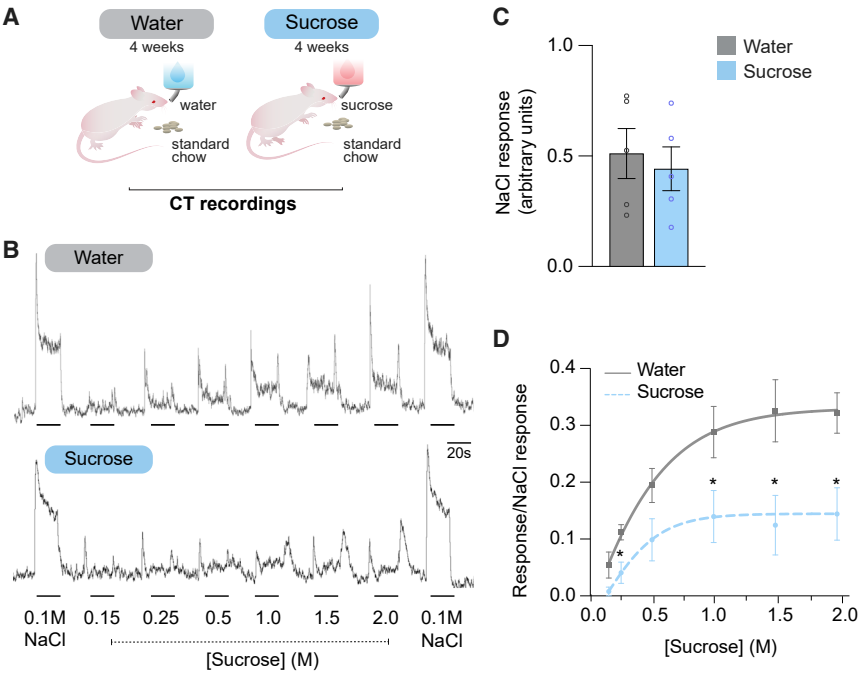

Figure 1. High dietary sucrose suppresses CT responses to sucrose but not NaCl

(A) Dietary treatments for adult rats fed standard chow with water (water) or with 30% (0.88 M) liquid sucrose (sucrose) for 4 weeks. See Figure S1 for liquid intake data.

(B) Representative integrated CT recordings from water and sucrose animals.

(C) Quantification of CT responses to NaCl from water (n = 5) and sucrose (n = 5) animals.

(D) Quantification of relative responses to different concentrations of sucrose (normalized to 0.1 M NaCl responses) in the water (n = 5) and sucrose (n = 5) groups. *p ≤ 0.05.

Data are mean ± SEM in all figures.

when sucrose was removed from the diet. Data show that sucrose consumption selectively reduced responses of the peripheral nerve to sucrose but not to other sugars, taste qualities, or sensory modalities. This reduction was not related to the amount of sucrose intake and returned to control values after the removal of sucrose from the diet. Whereas no effects on general FP structure, TB innervation, size, or total cell number were observed, there was a reversible decrease in the number of cells expressing PLCβ2, a marker of type II cells.[24] Thus, the peripheral taste system shows robust, specific, and reversible plasticity in response to alterations in dietary sucrose. The results reinforce the long-demonstrated existence of the diet-induced plasticity of the gustatory system[25] and demonstrate that refined sugars affect not only metabolism but also the sensory system.

RESULTS

To address how the consumption of high levels of dietary sucrose affects the peripheral taste system, adult male Sprague Dawley rats were given 30% (0.88 M) sucrose as their only source of liquid, while controls remained on water for 4 weeks. Both groups had free access to standard lab chow (Research Diets 5001LD) throughout (Figure 1A, study 1; water, n = 5; sucrose, n = 5). This concentration was chosen based on the rat preference for it[26,27] and on previous studies on dietary sucrose in rats[6] and in flies.[20]

In study 1, we recorded CT nerve responses to anterior tongue stimulation with 6 concentrations of sucrose (0.15–2.0 M). Despite drinking ~1,000 g of sucrose across the 4 weeks (Figure S1A), body weight did not differ between groups at the time of recording (water, 334.4 ± 7.59 g; sucrose, 341.6 ± 12.48 g; one-way ANOVA, $F_{(1,8)} = 0.24$, $p = 0.64$). Figure 1B shows representative integrated CT responses to sucrose from rats in water and sucrose groups. Each sucrose series was bracketed by delivery of 0.1 M NaCl. Responses to NaCl were similar in magnitude between groups (Figure 1C; one-way ANOVA, $F_{(1,8)} = 0.23$, $p = 0.64$), and all data were normalized to this internal standard. The CT response magnitude increased with higher sucrose concentration in both groups (Figure 1D; two-way mixed-model ANOVA; main effect of concentration, $F_{(2.140,17.120)} = 21.87$, $p < 0.001$). However, the sucrose response was substantially reduced in the sucrose versus water group (Figure 1D; two-way mixed-model ANOVA; main effect of group, $F_{(1,8)} = 9.12$, $p = 0.02$; group × concentration, $F_{(2.140,17.120)} = 2.89$, $p = 0.08$; Bonferroni post-test, $p < 0.05$). In addition, the CT response to sucrose plateaued at 1.0 M for the water group but at 0.5 M for the sucrose group. The data show that consumption of sucrose for 4 weeks lowers the response magnitude of the peripheral sensory nerve to sucrose stimuli, consistent with a previous study[6] and with findings in invertebrates.[20,22,23]

Restoration in CT responses to sucrose with the removal of sucrose diet exposure

To determine whether the effects of sugar exposure on the sensory nerve were persistent or transient, we replicated the design of study 1 but added a "recovery" group. Animals in this group were given regular chow with 30% sucrose water for 4 weeks (as the sucrose group) followed by an additional 4 weeks of regular chow and water (as the water group) (Figure 2A, study 2; water, n = 7; sucrose, n = 7; recovery, n = 6). Consistent with study 1, body weight was similar between groups at the time of recording (water, 425.17 g ± 9.25; sucrose, 442.20 g ± 8.96; recovery, 429.63 g ± 9.79; one-way ANOVA, $F_{(2,17)} = 0.94$, $p = 0.41$), but this cohort drank more sucrose than rats in study 1 (Figure S1). This is partly because animals in study 2 needed to be staggered more than in study 1 (as we only record from one rat per condition per day), and thus recordings were spread out over a wider range of ages (though all groups were age-matched within each study).

Figure 2B shows representative integrated CT responses to sucrose from rats in each group. Responses to NaCl were again used as a standard stimulus and did not differ across groups (Figure 2C; one-way mixed-model ANOVA, $F_{(2,17)} = 0.14$, $p = 0.87$).

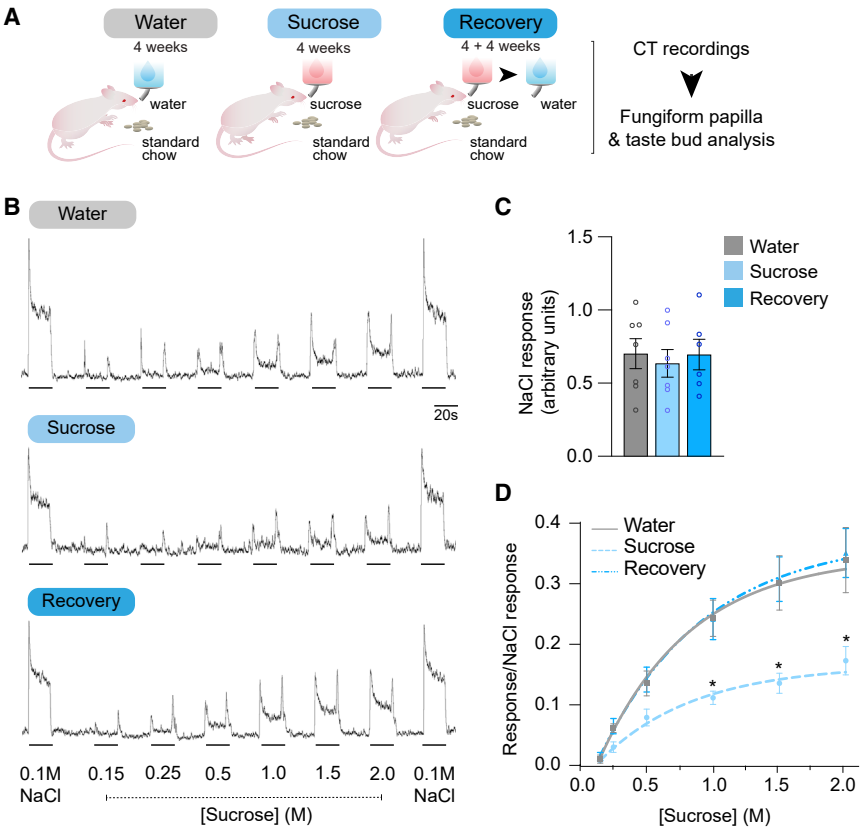

Figure 2. The effects of high sucrose exposure on CT responses are reversible

(A) Dietary treatments for adult rats fed standard chow with water (water) or sucrose (sucrose) for 4 weeks, or sucrose for 4 weeks and then water for 4 weeks (recovery). See Figure S1 for liquid intake data.

(B) Representative integrated CT recordings to NaCl and sucrose from water, sucrose, and recovery animals.

(C) Quantification of CT responses to 0.1 M NaCl across water (n = 7), sucrose (n = 7), and recovery (n = 6) groups.

(D) Quantification of relative responses to different concentrations of sucrose (normalized to 0.1 M NaCl responses) in the water (n = 7), sucrose (n = 7), and recovery (n = 6) groups. *p ≤ 0.05. See Figure S2 for sucrose off-response and Figure S3 for other sensory modalities.

We recorded CT activity to lingual stimulation with increasing sucrose concentrations in water, sucrose, and recovery groups and found higher CT responses to increasing sucrose concentrations in all groups (Figure 2D; two-way mixed-model ANOVA; main effect of concentration, $F_{(1.559,26.507)} = 98.10$, $p < 0.001$). Although the CT response to sucrose was nearly identical in water and recovery groups, it was reduced in the sucrose group (Figure 2D; two-way mixed-model ANOVA; main effect of group, $F_{(2,17)} = 8.31$, $p < 0.01$; group × concentration interaction, $F_{(3.18,\ 26.5)} = 4.78$, $p < 0.001$; Bonferroni post hoc water versus sucrose, 1.0, 1.5, and 2 M, $p < 0.03$). These data replicate the primary effect of the high-sucrose diet on the CT response from study 1 and demonstrate that the sensory deficit returns to control levels after the removal of sucrose from the diet.

A characteristic of CT responses to sucrose is that a neural discharge, called an "off-response," occurs when the sucrose is rinsed from the tongue using water.[28] This is only elicited after a few stimuli and is thought to be a characteristic of sucrose-sensitive receptors. Here, off-responses occurred when sucrose stimuli were rinsed from the tongue in sucrose and water groups (Figure S2; two-way mixed-model ANOVA; main effect of response, $F_{(1,17)} = 118.96$, $p < 0.001$). Despite showing a reduced response to all sucrose stimuli, the off-response was larger in recordings from sucrose rats compared with water controls (Figure S2; two-way, mixed-model ANOVA; main effect of group, $F_{(2,17)} = 3.65$, $p = 0.05$; group × response interaction, $F_{(2,17)} = 12.53$, $p < 0.001$; Bonferroni post hoc multiple comparison water off-response versus sucrose off-response, $p < 0.01$) but similar between the water versus recovery groups.

CT responses to other sugars, Na saccharin, and other taste qualities and modalities

To address the specificity of these diet-induced reductions, we recorded the CT response to other sugars and Na saccharin and to stimuli representing the qualities of bitter, sour, and umami. These stimuli were presented after the sucrose concentration series and were preceded and followed by stimulation with 0.1 M NaCl to ensure the stability of the CT recordings.

Examples of integrated CT responses to 0.1 M NaCl, 1 M glucose, 1 M maltose, 1 M lactose, 0.01 M Na saccharin, and 1 M fructose demonstrate similar responses to sugars and Na saccharin across groups (Figure 3A). The average CT response to each of these stimuli was similar across all groups (Figure 3B; two-way mixed-model ANOVA; no main effect of diet, $F_{(2,14)} = 1.90$, $p = 0.19$). Note that the response to 1 M sucrose from Figure 2 is included for ease of comparison. Thus, whereas consumption of a high-sucrose diet decreased the response to sucrose across concentrations, it did not alter the response to these other sugars.

We next examined the integrated CT response to chemicals representing a range of taste qualities: 0.04 M quinine HCl (bitter), 0.01 M citric acid (sour, organic acid), 0.01 N HCl (sour, inorganic acid), or 0.05 M monosodium glutamate (MSG; umami) (Figure 4). There were no differences in the relative response magnitudes to citric acid or umami stimuli (Figure 4B). However, the response to quinine HCl in the sucrose versus water group (Figure 4B; two-way mixed-model ANOVA; main effect of group, $F_{(2,16)} = 4.54$, $p = 0.03$; group × quality interaction, $F_{(3.649,29.189)} = 2.69$, $p = 0.06$; Bonferroni post-test sucrose versus water, $p = 0.05$) and to 0.01 N HCl (Figure 4B; sucrose versus water, $p = 0.03$) was higher. Thus, while responses to sucrose were substantially reduced (Figures 1 and 2), responses to bitter and inorganic acid stimuli were slightly increased by high dietary sucrose. Although we cannot exclude changes in CT responses at other concentrations, these data indicate the selective effects of elevated sucrose exposure on the peripheral taste system.

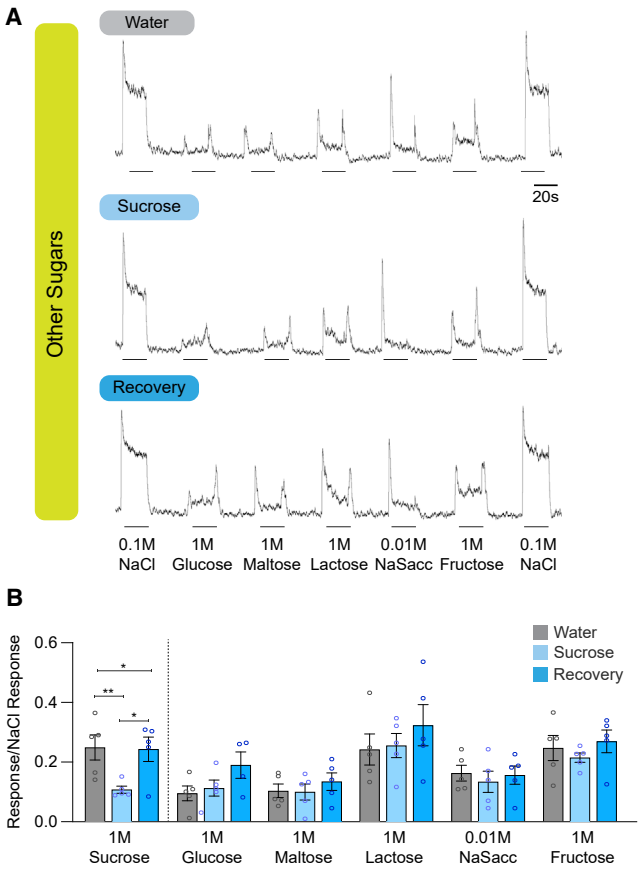

Figure 3. High sucrose consumption does not affect CT responses to other sugars

(A) Representative integrated CT recordings from other sugars and Na saccharin from a water, sucrose, and recovery rat.

(B) Quantification of relative responses (normalized to the 0.1 M NaCl response) to 1 M sugars and Na saccharin, compared with 1 M sucrose responses, in the water (n = 5), sucrose (n = 5), and recovery (n = 5) groups. **p ≤ 0.01, *p ≤ 0.05.

The CT nerve also responds to tactile and thermal stimulation.[29–32] To investigate whether high-sucrose diet treatment affected these sensory modalities, we stimulated the tongue with cold and touch (lightly stroking with a small brush) at the end of each recording session. No differences in the integrated CT nerve responses to these stimuli across dietary treatments were found (Figure S3). In concert, our neurophysiological data demonstrated that the effect of sucrose exposure on CT nerve responses was modality specific for chemosensation and for gustatory responses to sucrose, but not to other sugars or Na saccharin. In addition, the absence of effects on the CT response to cold or to touch demonstrates the integrity of the CT nerve during the long recordings.

Effects on anterior tongue taste organs: FPs and TBs

We reasoned that the effects of sucrose exposure on CT responses to sucrose could arise from alterations in the anatomical structures involved in the detection and transduction of taste signals. To test this hypothesis, we collected the tongues of rats used for CT nerve recordings in study 2 and quantified the number and shape of FPs as well as TB innervation, diameter, and cell subtypes.

FPs on the anterior tongue, sampled from the midsections of the one-half tongue, were analyzed in H&E-stained sections (Figures 5A and 5B). As described previously,[15] FPs can be divided into types I, II, and III (Figure 5B). Type I is characterized by normal papilla morphology with an apical TB. Type II and type III, in contrast, have an altered FP morphology; whereas type II FPs still contain the remnants of TB cells, type III FPs lack any TBs. When quantified (STAR Methods), the majority of FPs were type I, consistent with the literature,[33] and there were no differences in the distribution of type I, II, or III across groups (Figure 5C). Furthermore, the total number of FPs was similar across groups (Figure 5D). Thus, sucrose consumption did not perturb the overall number or the relative distribution of FP types.

An alternative possibility is that reduced CT responses to sucrose arise from changes in the number, size, morphology, or innervation of TBs within FPs. To examine this, we first used antibodies against markers of differentiated rat TB cells—cytokeratin 18 (K18), 19 (K19), and 8 (K8), defined here as K18+ cells—and measured TB diameter (Figures 6A and 6B). There were no group differences in TB diameter (Figure 6C). We also used antibodies against the purinergic 2X receptor 3 (P2X3) to label CT nerve afferents[34,35] and assess TB innervation (STAR Methods). Quantification of confocal images (Figure 6D) revealed no changes in the volume occupied by K18+ cells (Figure 6E) or P2X3+ afferents (Figures 6F and 6G) to the TB. Thus, changes in CT responses do not result from variations in TB size or innervation. This indicates that the FP taste organs remain intact following prolonged exposure to high sucrose.

In the tongue, sucrose is detected by type II taste cells via activation of G protein-coupled receptors (GPCRs); these cells also sense bitter and umami stimuli.[36–38] To examine the potential effects of sucrose on this population of cells, we used antibodies against phospholipase β2 (PLCβ2), a transducer molecule downstream of the taste receptors. We also co-labeled this tissue with an antibody to K18 to label all TB cells (Figure 7A). Although the total number of K18+ cells was unchanged (Figure 7B), the number of PLCβ2+ cells was reduced in the sucrose versus water group (Figure 7C; one-way ANOVA, $F_{(2, 105)} = 5.48$, $p = 0.005$; Tukey's multiple comparisons post hoc water versus sucrose, $p = 0.006$) but remained similar between water and recovery groups (Figures 7C–7D′).

DISCUSSION

Data here demonstrated that chronic high-sucrose exposure has large but selective effects on the rat peripheral taste system (Figure 7E). First, only the neurophysiological responses of the CT nerve to chemical stimuli were changed, while those to touch and cold stimuli remained intact. Second, among chemical stimuli, only responses to sucrose were decreased, whereas those to other sugars, organic acid, and umami were unchanged or only slightly increased. Third, there was a decrease in the numbers of PLCβ2+ (type II) cells but no differences in FP structure, TB size or overall cell number, or volume of innervation. Notably, both the effects on the CT nerve responses and on PLCβ2+ taste cell number recovered to control values after removing sucrose from the diet. Thus, the marked effects of sucrose exposure

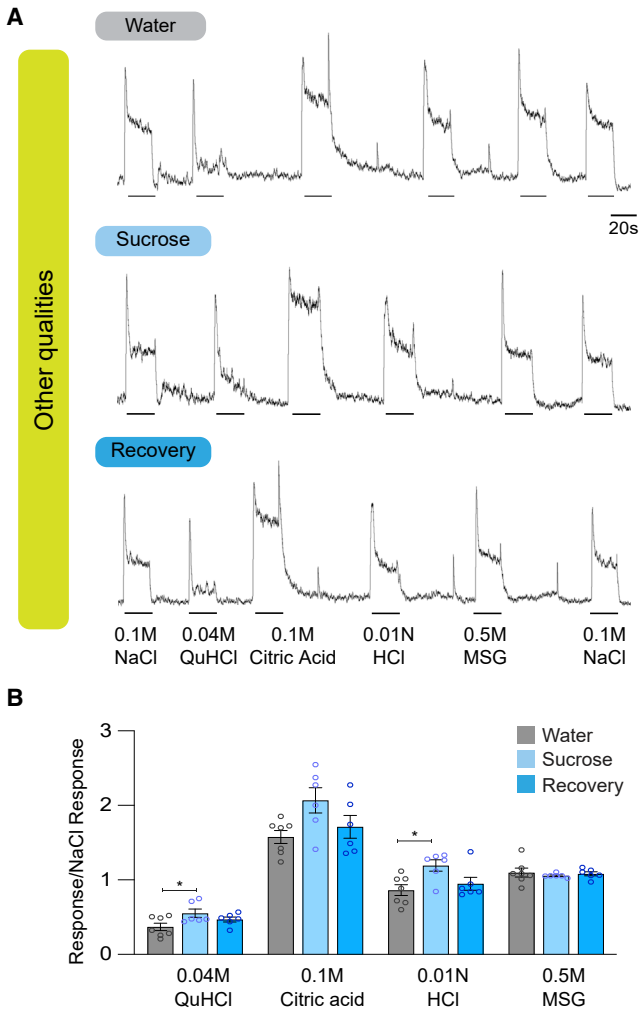

Figure 4. High-sucrose diet consumption has small effects on CT nerve responses to bitter and inorganic acid

(A) Representative integrated CT recordings to 0.1 M NaCl and bitter, sour, and umami taste stimuli, from water, sucrose, and recovery rats.

(B) Quantification of relative responses to bitter, acid, and umami stimuli, normalized to 0.1 M NaCl, in the water (n = 7), sucrose (n = 6), and recovery (n = 6) groups. *p ≤ 0.05.

are not due to an acquired pathology in the taste system, but rather to particular neurobiological sensory changes that occur in response to diet and that are reversible.

Selective effects of diet on peripheral responses to sucrose

Because we recorded directly from the CT nerve, the observed effects of diet on sucrose responses must arise from alterations in sensory receptor mechanisms rather than sensory neuronal processing or integration. The absence of effects on the responses to other sugars in sucrose rats, as well as other taste qualities and somatosensory modalities—with the exception of the small increases in QuHCl and in HCl responses (although the citric acid there is near significance, p = 0.06)—suggests that diet selectively impacts sucrose responses, although we cannot exclude that changes could occur at other concentrations.

These findings are largely consistent with a recent study that described lower CT responses to 1.0 M sucrose, but equal responses to glucose, in female rats fed regular chow with 30% liquid sucrose for 40 days.[6] However, unlike our findings that CT responses were decreased across a wide range of sucrose concentrations (Figures 1 and 2), McCluskey et al.[6] reported no differences at 0.01 and 0.5 M sucrose or with QuHCl and HCl. Furthermore, this study measured a decrease in CT responses to NaCl after sucrose exposure, while we did not find any differences across groups in both study 1 and 2. As the concentration of dietary sucrose was identical, these differences could arise from sex or the longer sucrose exposure (40 versus 28 days). To this end, exposure to 0.3 M sucrose for 3 days reduced the CT responses to 0.06 M sucrose without affecting responses to NaCl or other stimuli.[39] It is also worth noting that 30% dietary sucrose also selectively decreased taste responses to a wide range of sucrose concentrations in *D. melanogaster* flies,[20–22,40] suggesting that this primary effect is conserved.

Thus, the combined evidence from studies here and prior work indicates that chronic high dietary sucrose exposure selectively affects the sensory mechanisms responsible for the sensation of sucrose. What may contribute to this specificity? The differences in the CT sucrose response concentration curve between sucrose and water groups argue that this reduction could arise from a lower number of sucrose receptors. This is because the shape of this curve depends on the number of receptor sites bound, which increases in response magnitude with concentration until all the receptor sites are occupied.[41] To reconcile this hypothesis with the absence of effects on other sugars tested, independent sensory mechanisms would have to exist for different sweet-tasting chemicals. Recordings from single rat CT fibers indicate that those strongly activated by sucrose were distinct from those responding to fructose, maltose, and glucose.[42] Furthermore, despite early work supporting the idea that a single sweet taste receptor, the heterodimeric T1R2/T1R3 GPCR, was responsible for all responses to sugars,[36] evidence in support of multiple mechanisms and pathways for sweet taste sensation has accumulated.[43] For example, a parallel sweet-sensing pathway based on glucose transporters, sodium-glucose transporters, and metabolic enzymes may detect different types of sweet stimuli,[43,44] and this may account for the remaining CT responses to high sucrose in T1R3, T1R2, and TRPM5 knockout mice.[36,45,46] In this light, the specific effects of high dietary sucrose on CT nerve responses to sucrose, but not other sugars described here, provide further evidence for multiple sweet taste receptors or pathways. They also highlight how much we still do not know about the nature of sweet taste reception.

Effects of a sucrose diet on the anatomy of the taste structures

Besides examining neurophysiological differences in response to the sucrose diet, we also studied the FP and TB. The diameter and innervation volume, as well as the number of the K18+ cells, were unchanged by diet. These findings are consistent with the specificity of effects and maintenance of CT responses to a range of other chemical, tactile, and temperature stimuli. In contrast, the number of PLCβ2+ cells was lower in sucrose animals. A decrease in PLCβ2+ cells in the absence of an overall reduction in TB size

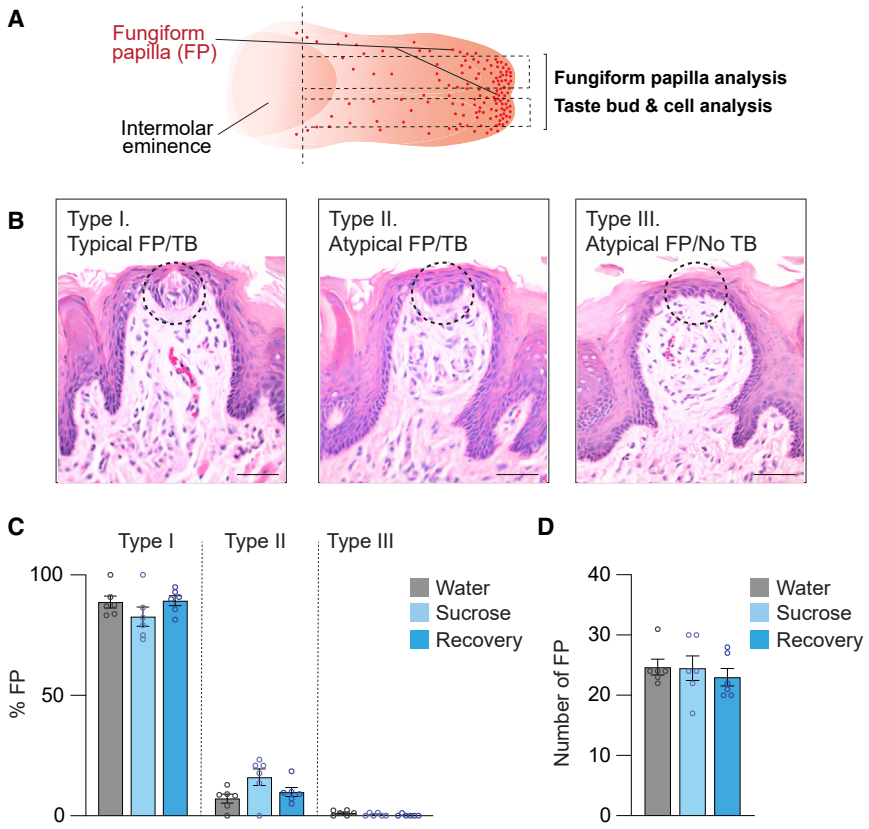

Figure 5. High-sucrose diet has no effect on the fungiform papilla types or numbers

(A) The region of the anterior tongue (within hatched lines) used to analyze the fungiform papillae (FPs) and taste buds (TBs).

(B) Morphological categories of FP types (I, II, and III) studied with H&E staining. Scale bars, 50 μm.

(C) Quantification of FP types in water (n = 6), sucrose (n = 6), and recovery (n = 6) rats, with 17–31 FP analyzed for each tongue.

(D) Total number of FPs in an 800-μm portion of the tongue, averaged for water, sucrose, and recovery diet group (n = 6 tongues for each group).

and cell number could be due to a change in the overall composition of the TB or the limited sensitivity of our morphological approaches. Furthermore, cells labeled by PLCβ2 are sensitive to either bitter, umami, or sweet (type II),[9,33,47–49] but we detected a small increase in bitter and no change in umami responses. Although this could also indicate a shift in the overall specification of type II cells, it could also be due to the limited concentrations and compounds tested for these stimuli. Finally, a likely possibility is that these reductions in PLCβ2+ cells only play a modest role—or perhaps even no role—in the reduction in CT responses to sucrose. Instead, this effect could be caused by changes in the expression, levels, or function of the receptors or the signaling molecules that transduce taste, as shown in flies.[21,50]

We also observed that changes in PLCβ2+ cells recovered when sucrose was removed from the diet. As both the papilla epithelium and TB cells continuously turn over (3–30 days, average of 10 days),[51,52] this recovery is not surprising. Type II cells have a reported half-life of 8 days,[53] and "newborn" cells that enter the TB can complete differentiation within 2–3 days,[54] well within the 4 weeks of our recovery experiment. Importantly, neurophysiological and TB deficits recover even after major disruptions in rodents (e.g., complete loss of TBs caused by pharmacologic inhibition or mutations in the Hedgehog signaling pathway[15,55]). Nonetheless, our findings are important for several reasons: first, they are the first to show recovery from sucrose exposure in the mammalian gustatory system; second, they fit with existing evidence of plasticity in taste,[25] which has key implications for the acceptance of foods with lower levels of sugar and sodium.[56]

Diet-induced plasticity in the taste system

In regard to understanding the impact of diet composition on taste, attention in the chemosensory field has long been drawn to the effects of altered intake of NaCl.[56] Due to the global rise in obesity more recently, this interest has shifted to also studying the effects of sugars and fat on taste. This literature is in the early stages of development, and studies directly comparable to those done here using high dietary fat have yet to be done. However, there is some evidence that fat, such as sugar and salt, may also alter peripheral taste responses. For example, in mice, 6–8 weeks of a high-fat diet reduced the *ex vivo* calcium responses to sucrose and saccharin of isolated TBs[57,58] and lowered the expression of PLCβ2 in the circumvallate TB.[58,59] Similar to our findings, consumption of a high-energy (48% fat, 17% sucrose) diet had no effect on the number of FPs and taste pores; notably, there was no effect of diet on psychophysical detection threshold tests for NaCl and sucrose (0.6–0.0005 M) in these animals, but suprathreshold intensity and detection threshold were not measured.[60] Importantly, it will be critical to study the effects of sucrose exposure beyond the FP and CT nerve, such as the circumvallate and foliate papillae on the posterior tongue (glossopharyngeal nerve), the TB on the soft palate (greater superficial petrosal nerve),[61] and the TB in the pharynx and larynx (superior laryngeal nerve).

Although careful studies comparing the effects of dietary fat versus sugar are needed, the limited data are consistent with inverse correlations between levels of dietary sugar and fat and the peripheral sensation of sweet taste in humans.[62–64] Thus, systematic evaluation of the effects of fats and sugars on taste systems and mechanistic studies of diet-induced sensory alterations are needed to improve nutrition and eating patterns in humans.[7,8] In this context, it will be essential to differentiate the effects of dietary exposure to sugar and fat from those of weight or fat accumulation, as circulating signals such as leptin or glucose can influence peripheral and central gustatory-evoked responses.[65–67] Indeed, although no differences in body weight were found across experimental groups here, we cannot rule out the possibility that some of the effects observed could arise from adiposity and/or metabolism. It is also worth

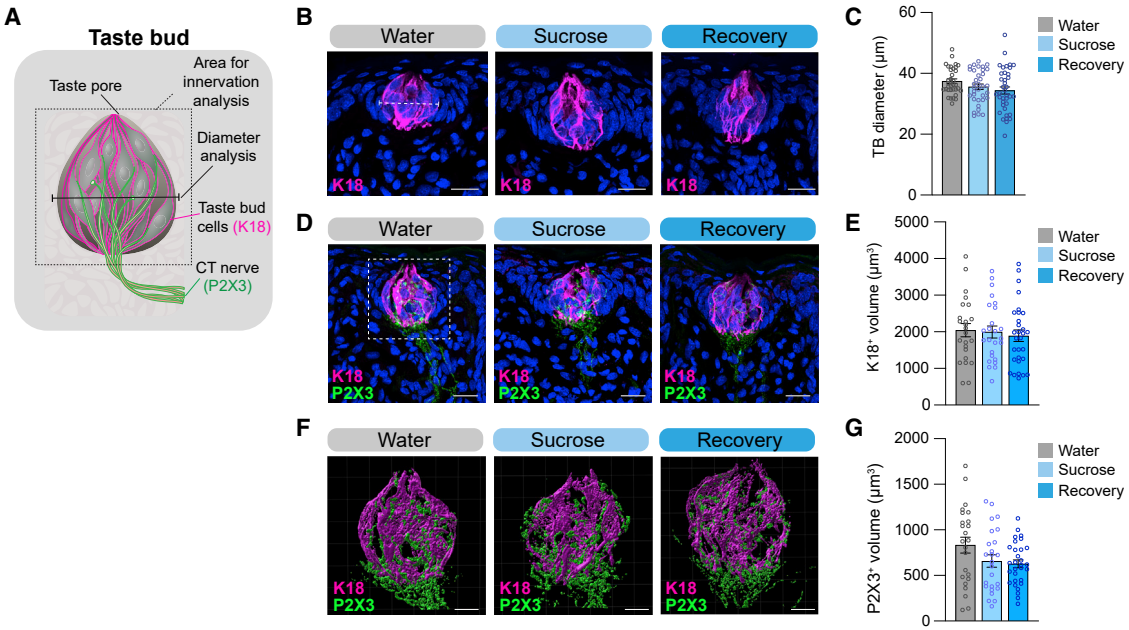

Figure 6. High dietary sucrose does not change TB size and innervation

(A) Regions used to quantify the TB diameter and CT innervation and the location of cytokeratin 18 (K18)+ cells and the P2X3+ CT nerve.

(B) Representative confocal maximum-intensity projections where the TB cells were labeled with K18 antibody (magenta) and DAPI (blue), from water, sucrose, and recovery animals. The hatched line in the water image represents the point of TB diameter measure. Scale bars, 20 μm.

(C) Quantification of TB diameter from water, sucrose, and recovery groups. n = 6 tongues for each dietary group, with 4–8 TBs analyzed per tongue.

(D) Representative confocal maximum-intensity projections from water, sucrose, and recovery rats where the CT nerve was labeled with P2X3 antibody (green) and the TB cells with K18 antibody (magenta) and DAPI (blue). The dotted box in the water image represents the region used to measure TB volume based on K18+ cells. Scale bars, 20 μm.

(E) Quantification of TB volume based on the K18+ cell area from water, sucrose, and recovery animals. n = 5–6 tongues for each group, with 3–7 TBs analyzed per tongue.

(F) Surface renderings in a 10-μm square region of confocal images showing the volume of CT innervation, as labeled by P2X3 antibodies, in water, sucrose, and recovery rats. Scale bars, 20 μm.

(G) Quantification of CT innervation based on the volume of P2X3+ cells from water, sucrose, and recovery animals. n = 5–6 tongues for each dietary group, with 3–7 TBs analyzed per tongue.

noting that the effects of intermittent sucrose exposure could differ from those of continuous exposure and that the threshold of sucrose needed to reduce sensory responses in mammals is currently unknown. We observed that despite differences in sucrose intake between our two studies (Figure S1), the reduction in sucrose CT responses was comparable. This could suggest that a minimum amount of sucrose exposure is needed to lower CT responses, after which there is no further effect. Our work in flies suggests that sensory alterations occur independently of fat accumulation and develop at 15%–30% sucrose, fructose, and glucose within 2 days but worsen with longer exposures;[20,21,40,50] we also found that diet-induced taste plasticity drives higher eating and obesity in flies,[20,40] but mammalian studies have yet to address this question.

Summary and future directions

Here, we examined the effects of a high sucrose diet on CT nerve responses to an array of chemicals, compared chemosensory with touch and temperature modalities, and assessed FP and TB changes. We show a selective and reversible effect of sucrose exposure on peripheral CT nerve responses and on PLCβ2+ type II cells. Although the literature is replete with the discussion of dietary sucrose as related to human disease and altered metabolism,[4,68] data showing the effects on the peripheral taste system per se are scarce. Thus, our results form an essential basis for further studies and behavioral investigations. When combined with the growing knowledge about gut-brain and metabolism-sensory interactions, our findings can help reveal the multifaceted neurobiological consequences of sucrose consumption.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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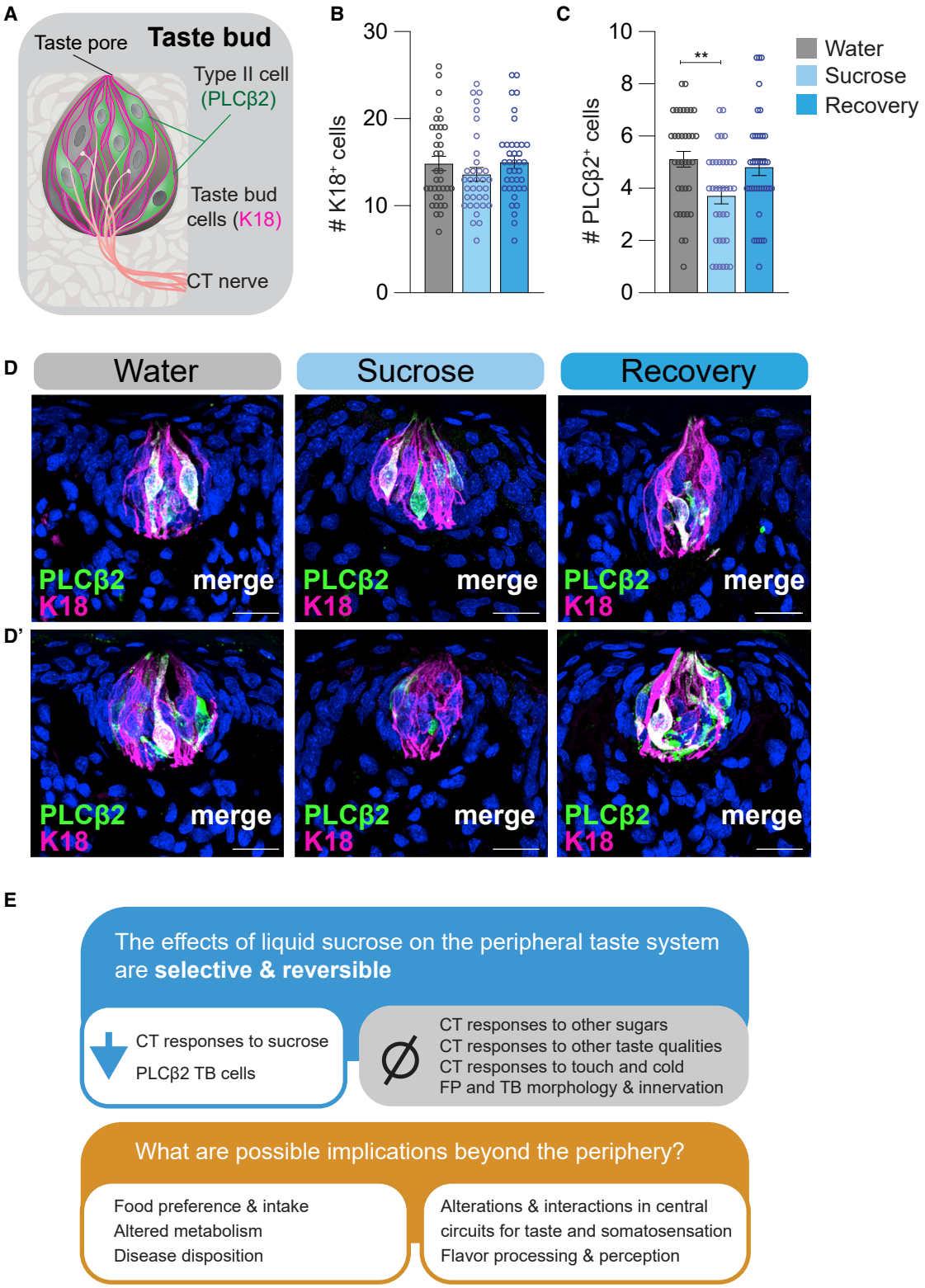

Figure 7. The number of PLCβ2+ (type II) taste cells is lower in sucrose but not recovery animals

(A) Schematic of the TB with type II cells labeled with PLCβ2 (green) and all taste cells labeled with K18 (magenta).

(B and C) Quantification of the number of (B) K18+ cells and (C) type II PLCβ2+ cells in a 10-μm section of the tongue, from water, sucrose, and recovery animals. n = 6 tongues for each group; 4–8 TBs were analyzed for each tongue. **p ≤ 0.01.

(legend continued on next page)

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2022.07.063.

A video abstract is available at https://doi.org/10.1016/j.cub.2022.07.063#mmc3.

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AUTHOR CONTRIBUTIONS

Conceptualization, R.M.B., M.D., C.R.F., and C.M.M.; animal handling and feeding, H.D. and C.R.F.; electrophysiology, R.M.B., with assistance from C.M.M., H.S., and I.V.; tissue preparation, imaging, and analysis, M.D., C.M.M., H.S., and I.V.; data analysis, R.M.B., M.D., C.R.F., C.M.M., H.S., and I.V.; supervision, R.M.B., M.D., C.R.F., and C.M.M. R.M.B., M.D., C.R.F., and C.M.M. wrote the manuscript with ongoing discussion, input, and editing from H.S. and I.V. All authors approved the final submission.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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(D and D′) Maximum-intensity confocal projections of K18+ (magenta) and PLCβ2+ (green) cells from two representative rats per dietary condition (water; sucrose; recovery). Scale bars, 20 μm.
(E) Summary of the selective and reversible effects of high dietary sucrose on the magnitude of CT responses to sucrose stimuli and on the number of PLCβ2+ cells (pink). Implications are presented for future questions beyond the peripheral taste system (orange).

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-cytokeratin K 8+ 18+ 19	Abcam	Cat#ab41825; RRID: AB_736438
Rabbit anti-P2X3	Alomone labs	Cat#APR-016; RRID: AB_2313760
Rabbit anti-PLCβ2	Santa Cruz Biotechnology	Cat#sc-206; RRID: AB_632197
Donkey anti-rabbit Alexa 488	Invitrogen	Cat#A21206; RRID: AB_2535792
Donkey anti-Mouse Alexa 568	Invitrogen	Cat#A10037; RRID: AB_2534013
Chemicals, peptides, and recombinant proteins		
Sucrose	Sigma-Aldrich	Cat#84097
QuHCl	Sigma-Aldrich	Cat#Q1125
Citric acid	Sigma-Aldrich	Cat#C0759
HCl	Sigma-Aldrich	Cat#H9892
MSG	Sigma-Aldrich	Cat#1446600
Glucose	Sigma-Aldrich	Cat#D9434
Maltose	Sigma-Aldrich	Cat#M5885
Lactose	Sigma-Aldrich	Cat#17814
NaSaccharin	Sigma-Aldrich	Cat#47839
Fructose	Sigma-Aldrich	Cat#F0127
NaCl	Sigma-Aldrich	Cat#S7653
VECTASHIELD mounting medium with DAPI	Vector Laboratories	Cat#NC1601055
Experimental models: Organisms/strains		
Male Sprague-Dawley Rat	Envigo; Indianapolis, IN	aged 50-55 days on arrival
Software and algorithms		
Spike 2	Cambridge electronic Design	RRID: SCR_00093
Huygens professional	Scientific Volume Imaging	RRID: SCR_014237
Fiji/ImageJ	https://fiji.sc/	RRID: SCR_002285
Imaris 9.7	Oxford Instruments	RRID: SCR_007370
SPSS Statistics 26	IBM	RRID: SCR_019096
Prism 9	GraphPad	RRID: SCR_002798

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Monica Dus (mdus@umich.edu).

Materials availability

No new reagents or materials were generated by this study. The reagents used are listed in the key resources table and are commercially available.

Data and code availability

- Microscopy data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animal use and care procedures were in accordance with National Institutes of Health guidelines and University of Michigan Animal Care and Use Committee approved protocols. Male Sprague-Dawley rats, approximately 55-days old, were housed individually in a climate-controlled, reverse 12h light-dark cycle room, and given a 1-week acclimatization period before any experimental procedures. Rats weighed 199-212g initially and had free access to standard laboratory chow throughout the studies. Body weight and liquid intake per rat were measured weekly at the same time of day. Animals started their designated liquid exposure at staggered times in the study to ensure that neural recordings of each animal could be obtained after 4-5 weeks on the liquids. Animals had ad-lib access to food throughout and were not fasted prior to recordings. All recordings began approximately 3 hours into the dark cycle. The same animals were used for neurophysiology and tongue morphology and immunohistochemistry experiments. Male rats were studied to obviate any effects of female hormone cycling over the long time course of experiments. Based on the literature for male and female rodents we did not predict gender effects for peripheral nerve neurophysiology or tongue and TB studies.[12,15,55]

METHOD DETAILS

Experimental studies

We conducted two studies. Study One: to determine whether there would be peripheral, sensory afferent neurophysiological effects to stimulation of the tongue with sucrose concentrations, after long exposure of animals to a high sucrose liquid diet. This initial study provided a test of the concentration used for the high sucrose diet and the timing for diet exposure. Study Two: to determine if there were effects of the high sucrose diet to a broad range of taste stimuli and to other sensory modalities of touch and cold; and, to test for recovery from the long-term exposure effects of a high sucrose liquid exposure. In this study, we also studied the structure of FP and TBs, and taste bud cell types to learn whether the high sucrose diet affected the morphology of anterior tongue taste organs.

Study one

Animals were separated into two groups of similar average body weights (n=5 rats/group). Control rats (Water group) were given free access to regular drinking water. Experimental rats (Sucrose group) were given free access to only 30% (0.88M) sucrose liquid, made by dissolving sucrose (Sigma-Aldrich) in the same regular drinking water given to the Water group.

Study two

Animals (N=20) were separated into three groups of similar average body weights, with a total of 20 rats in the study. Control rats (n=7) were given free access to regular drinking water (Water group). Experimental rats (n=7) were given free access to only 30% (0.88M) sucrose liquid, made by dissolving sucrose (Sigma-Aldrich) in the same regular drinking water given to the water group (Sucrose group). A third group (n=6) was initially given free access to only 30% sucrose liquid for 4 weeks and then were given water for 4 weeks (Recovery group). No differences in body weight were observed between groups following dietary treatment.

Neurophysiological recordings from the chorda tympani nerve

Taste function was assessed by recording from the chorda tympani (CT) nerve, a branch of facial nerve VII, that innervates FP and TBs on the anterior tongue. Rats were anesthetized with pentobarbital sodium (50 mg/kg) via intraperitoneal injection, with supplemental doses given to maintain surgical anesthesia. As described in STAR Methods from our previous papers,[55,69] rats were placed on a heating pad to sustain body temperature and secured in a non-traumatic head holder[31] for surgery. The trachea was cannulated and both hypoglossal nerves were cut to prevent reflex tongue movement. The left CT nerve was exposed by a lateral dissection of the head, cut near its entrance to the tympanic bulla, and then freed to the branch point with the lingual nerve. The nerve was desheathed and placed on a platinum-iridium electrode with a reference electrode nearby. The tongue was gently extended for stimulus application. Multi-fiber neural activity from the CT was amplified using a Grass P511 preamplifier, displayed on an oscilloscope, and monitored by an audio amplifier. The amplified neural activity was passed through an integrator circuit[69] and stored and digitized in the Spike 2 version 4 program (Cambridge Electronic Design). Investigators were blind to groups during recording and data analysis.

Stimuli, stimulation protocol, and response measures

Reagent-grade stimuli dissolved in distilled water were used. Stimulus solutions (5 ml) were applied via syringe to the anterior tongue that was gently extended and secured to the recording table. Tactile stimuli consisted of lightly stroking the anterior tongue quadrant three to six times over about 5 s with a wooden rod. Water at 4°C was used for cold stimulation. In Study Two, three sets of chemical stimuli were used. The first set, for sucrose concentrations, consisted of increasing concentrations of sucrose (0.15, 0.25, 0.50, 1.00, 1.50 and 2.00 M). A second set, to represent 'taste qualities', was 0.04 M QuHCl (bitter), 0.10 M citric acid (sour), 0.01N HCl (sour), and 0.50 M MSG (monosodium glutamate - umami) stimuli. The third set, defined as the "sweet series", included 1.0 M glucose, 1.0 M maltose, 1.0 M lactose, 0.01 M Na Saccharin, and 1.0 M fructose. These concentrations were chosen because studies show that they elicit robust taste responses in recording procedures similar to those used here. Of note, Saccharin is often thought of as tasting "bitter" as well as sweet. However, the compound tested here, Na Saccharin, has not been shown to induce bitter responses during CT recordings.[70]

A solution of 0.10 M NaCl was used as a repeated, standard stimulus, and stimulus sets were bracketed by lingual stimulation with 0.10 M NaCl. Chemicals were reagent grade, and solutions were made with room temperature distilled water. Stimuli were applied to the tongue for 20 s and then the tongue was rinsed with distilled water for about 30 s until the baseline stabilized.

Response magnitudes for each stimulus were calculated by measuring the integrated neural response above baseline at 5 – 10 s after the stimulus was applied. The response value for each stimulus in a given set was then reported as an 'absolute' unit measure or as the integrated response value as a proportion of the mean integrated responses to 0.10 M NaCl that bracketed the set. Only sets bracketed by 0.10 M NaCl taste responses that differed in magnitude by $\leq$ 20% were included in the analysis. All three stimulus sets were repeated at least twice.

Due to the difference in delivery between somatosensory and chemical stimuli,[12,15,29,30,55,69,71–73] touch and temperature CT responses are reported as raw (not integrated) data per standard practice in the field.

Tongue morphology, papilla, and taste bud quantification, and immunohistochemistry

In Study Two, after recordings were completed from the CT nerve, tongues were dissected and prepared for further study. We collected anterior tongue and FP tissues. In the current work, our focus was on the anterior tongue, FP, and TBs innervated by the chorda tympani nerve.

Tissue preparation

As described in detail[29,55,69] the tongue seated on the mandible was dissected and fixed in 4% paraformaldehyde in PBS for 2 hours at room temperature. Tongues were then dissected from the mandible and fixed for an additional 1 hour. The anterior tongue was cut at the most rostral end of the intermolar eminence and both parts were fixed for an additional 3 hours. Tongues were washed in Phosphate Buffered Saline (PBS) and then dissected into three pieces: the anterior tongue had been cut at the most rostral end of the intermolar eminence, and was bisected down the midline into two pieces; the posterior tongue piece, which included the single circumvallate papilla, was trimmed to square the tissue and the excess ventral tissues of muscle and connective tissue. One anterior tongue piece from each rat was separated, and fixed overnight in 4% paraformaldehyde in PBS for subsequent embedding in paraffin and H&E staining. These anterior halves were serially sectioned at 8 μm in the sagittal plane. The other anterior tongue half from each rat was placed in 30% sucrose in PBS at 4°C overnight, for cryoprotection, and then frozen in O.C.T. on the following day. For this anterior half, frozen, serial sagittal sections at 10 μm were cut and mounted on slides for immunofluorescence.

Immunofluorescence and imaging

Immunofluorescence was performed as detailed in our published procedures.[15,55] Slides were air dried, washed in PBS, and then blocked for one hour in 20% donkey serum. Primary antibodies were: taste cell markers: mouse anti-cytokeratin 8+ 18+ 19+ (2A4) (ab41825, Abcam, 1:100); Rabbit anti-P2X3 (APR-016, Alomone labs, 1:5000); rabbit anti-PLCβ2 (Q-15) (sc-206, Santa Cruz Biotechnology, 1:500). Secondary antibodies were Alexa Fluor conjugates 488 or 568 (A21206 and A10037, respectively, Invitrogen, 1:500). Sections were mounted with VECTASHIELD mounting medium containing DAPI. Images were acquired with a Leica SP8 confocal microscope with a 93x objective at 0.33μm intervals. Confocal images were processed using Huygens deconvolution software and Fiji/ImageJ.

QUANTIFICATION AND STATISTICAL ANALYSIS

Quantification of the fungiform papilla and taste bud types

On each half tongue in hematoxylin and eosin serial, sagittal sections, we analyzed 800 μm of the middle tissue sections to exclude sections on the lateral lingual border or in the midline. We categorized three types of FP and TBs, described in detail in prior publications,[15,69] and briefly described here as TYPE I. Typical FP and Typical TB: contains a rectangular papilla with multilayered epithelium, a broad connective tissue core, and a single TB with an identifiable taste pore. TYPE II: Atypical FP and Atypical TB: the papilla is somewhat misshapen and the TB has reduced cells, without a taste pore. Type III: Papilla and No TB. The papilla is misshapen, has a pointed or conical apex, and has either no or extremely few TB cells. These categories are illustrated in Figure 5. Data are reported as the percentage of FP/TB Type I, II, or III, relative to full FP/TB counts (about 30 FPs per tongue).

Quantification of taste bud size

The confocal z-stack images of each anterior tongue slide stained with anti-K18 antibodies were examined for the presence of a taste pore to identify the middle plane of the TB. The diameter was quantified by using Fiji/Image J to calculate the width of the largest portion of the TB labeled by K18 antibodies.

Quantification of taste bud volume and CT innervation

The confocal and deconvoluted z-stack images of anterior tongue sections stained with anti-K18 and anti-P2X3 antibodies were loaded onto the Imaris 9.7 (Oxford instruments) software. For the TB volume, the ''Surface'' function with automatic detection was used to calculate the volume of K18+ cells. For CT nerve innervation, a square region was drawn 10 μm from the edge of the taste in four directions, centered in the middle of the TB. The ''surface'' function with automatic detection was used to calculate the volume of P2X3+ neurites in the square. For volume and innervation measurements, background subtraction was set to 8 μm for K18+ cells and 1 μm for P2X3+ neurites, but images were also checked visually to make sure background signals were uniformly excluded. Three to seven FP per tongue and five to six tongues per dietary condition were quantified.

Quantification of type II TB cells

To quantify the number of Type II cells, considered to have receptors for sweet, bitter, and sour stimuli,[36–38] confocal z-stack images of anterior tongue sections labeled with anti-K18 and anti-PLCβ2 antibodies and DAPI were loaded onto Fiji/Image J. The Cell Counter plugin was then used to count the number of K18+ and PLCβ2+ in each confocal slice by following the same cell across the stack using DAPI nuclear stain as reference. Four to eight TBs per tongue section were counted.

Statistics

For electrophysiology, SPSS Statistics 26 (IBM, USA) software was used to perform one-way ANOVA with Tukey's posthoc test for NaCl neurophysiological recordings data, two-way, mixed-model ANOVA followed by Tukey's posthoc test and Bonferroni pairwise comparison for neurophysiological recordings data and Kruskal-Wallis test for liquid intake data. For the FP and TB analyses, data were tested for normality, and One-way ANOVA analysis with Tukey posthoc test was then performed using Prism (GraphPad). Prism (GraphPad) software was used to make all graphs; data in figures are presented as Mean ± SEM. Significance was set at $p \leq 0.05$. Exact p values are listed in figure legends and results.