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The cellular mechanism for water detection in the mammalian taste system

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Initiation of drinking behavior relies on both internal state and peripheral water detection. While central neural circuits regulating thirst have been well studied, it is still unclear how mammals recognize external water. Here we show that acid-sensing taste receptor cells (TRCs) that were previously suggested as the sour taste sensors also mediate taste responses to water. Genetic silencing of these TRCs abolished water-evoked responses in taste nerves. Optogenetic self-stimulation of acid-sensing TRCs in thirsty animals induced robust drinking responses toward light even without water. This behavior was only observed when animals were water-deprived but not under food- or salt-depleted conditions, indicating that the hedonic value of water-evoked responses is highly internal-state dependent. Conversely, thirsty animals lacking functional acid-sensing TRCs showed compromised discrimination between water and nonaqueous fluids. Taken together, this study revealed a function of mammalian acid-sensing TRCs that provide a cue for external water.

Appetite represents an important basis of homeostatic regulation that drives animals to goal-oriented consummatory behaviors. These innate behaviors are finely controlled based on internal needs and external nutrient sensing[1,2]. For instance, thirsty animals engage in drinking behavior only when water is sensed at the periphery. Defining the central and peripheral neural logic underlying appetite is critical for understanding homeostatic regulations. In mammals, specific neural populations of circumventricular organs in the brain sense internal water balance and regulate water appetite[3–7], whereas how animals detect water at the periphery remains unexplored.

Oral sensation serves as an initial sensory checkpoint that evaluates nutrient factors from the external environment. The mammalian taste system detects essential nutrients, as well as toxic substances, through taste receptors and channels expressed in TRCs[8–11]. For example, low sodium is sensed by a single class of TRCs expressing the epithelial sodium channel ENaC. Knocking out of the ENaCα gene abolishes appetitive salt intake[12,13]. Similarly, the tastes of L-amino acids, sugars and bitter substances are recognized by dedicated receptors and TRCs on the tongue[14–17]. Acids are detected by a distinct set of TRCs expressing PKD2L1, a polycystic-kidney-disease-like channel[18–20]. In contrast to these basic tastes, it is still controversial whether the detection of water, another vital nutrient for the body, is mediated by the taste system in mammals.

Many decades of work have shown that invertebrates such as *Drosophila melanogaster* can sense water through a specialized taste cell population[21,22]. Recent studies have demonstrated that PPK28, a member of the DEG/ENaC family, is required for sensory responses to external water, as well as water-seeking behavior[23]. In vertebrate species such as frogs, sheep and cats, water has been shown to elicit electrophysiological responses in facial nerves innervating the oral cavity[24–26]. Moreover, water-induced responses have been reported in taste-related neurons of the nucleus of the solitary tract in rodents[27]. Although the underlying mechanisms is unknown, these studies suggested that water detection is, in part, encoded by the taste system.

RESULTS

We reasoned that if water is sensed as taste in mammals, at least two criteria should be met; first, taste responses to water should be mediated by specific cellular and molecular substrates in the taste bud and, second, activation of this pathway should encode a cue for external water. As an initial step to test these hypotheses, we employed *in vivo* extracellular recording from the chorda tympani taste nerves to explore water responses. By stimulating the tongue with various solutions, we observed robust nerve responses to deionized water, along with other basic tastants (**Fig. 1a**), demonstrating that water effectively activates the taste system.

How does application of deionized water induce action potentials in TRCs? The mammalian oral cavity is normally covered with a thin layer of saliva containing various ions and enzymes[28,29]. In our experiments, we used artificially reconstituted saliva made of the ionic components of normal saliva and found that washing out these ions with water generated robust nerve responses. These observations indicate that salivary ions play a key role in causing the responses. Therefore, we examined the effects of individual saliva components on water-induced responses. Intriguingly, we found that the responses to water were triggered when bicarbonate ions were present in the preceding solutions: switching from bicarbonate solution to water triggered robust nerve responses, whereas switching from bicarbonate-free saliva to water failed to induce responses (**Fig. 1b**). No other ions in artificial saliva induced taste responses when changed to water (**Fig. 1b** and **Supplementary Fig. 1a**), although high concentrations of phosphate caused minor responses (**Supplementary Fig. 1b**). Indeed, potassium bicarbonate evoked dose-dependent nerve responses when

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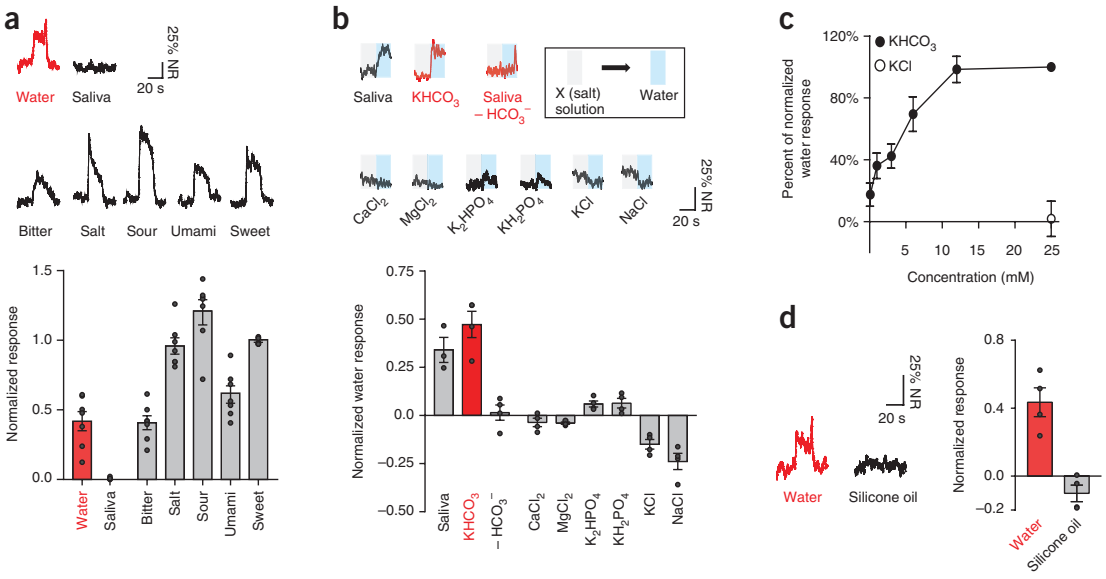

Figure 1 Water responses in the mammalian taste system. (**a**) Water elicits robust responses in chorda tympani taste nerves. Shown are representative integrated chorda tympani nerve responses to water and five basic tastants (upper) and their quantified data (bottom). Application of water evoked significant taste responses ($n = 7$ mice; $P = 0.0006$, water versus saliva). NR, normalized response. Tastants used were bitter (0.1 mM cycloheximide), salt (60 mM NaCl), sour (10 mM citric acid), umami (50 mM monopotassium glutamate plus 1 mM inosine monophosphate) and sweet (8 mM acesulfame potassium). Artificially reconstituted saliva solution (see Online Methods) was used as a base solution for all stimuli. (**b**) Effects of individual ion components on water responses. In representative traces (top), gray and blue shades denote each salt solution and water, respectively; the trace for saliva minus HCO_3^- was from a different animal. Average water responses elicited in different salt solutions were quantified (bottom). Removal of bicarbonate ions elicited water responses while application of water following saliva lacking bicarbonate ions or solutions of other ion species had no effect ($n = 3$ for saliva, $n = 4$ for other solutions; $P = 0.0286$, $KHCO_3$ versus saliva – HCO_3^-). (**c**) Dose dependence of water responses to potassium bicarbonate. Water induced larger responses with higher concentrations of potassium bicarbonate while it induced no response with potassium chloride ($n = 4$). (**d**) Washout of saliva with nonaqueous silicone oil did not induce response. Shown are representative traces to water and silicone oil (left) and quantification of responses (right, $n = 4$; $P = 0.0286$, water versus oil). Statistical significance was analyzed with two-tailed Mann-Whitney *U*-test. Values are means ± s.e.m.

switched to water while the same concentration of potassium chloride had no effect (**Fig. 1c**). Together, these results point out two important characteristics of taste responses to water: (i) the responses are induced by washout of saliva with water, mainly mediated by bicarbonate, and (ii) unlike in invertebrate water detection[23], osmolality change by itself is not the key determinant.

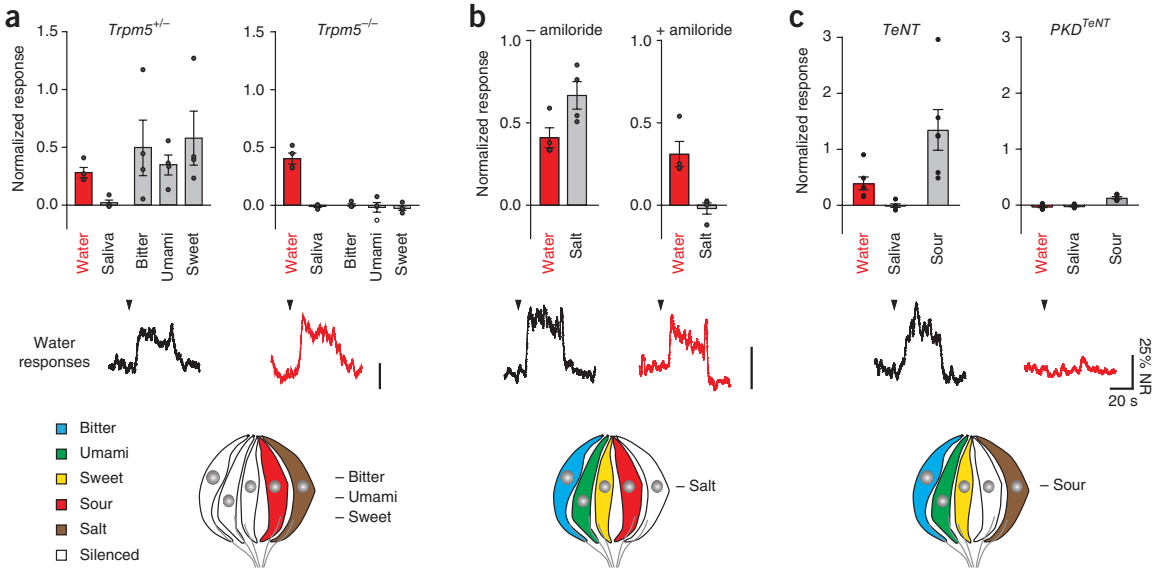

Figure 2 Water activates the acid-sensing taste pathway. (**a**) *Trpm5*$^{-/-}$ mice show no responses to bitter, umami or sweet tastants. However, they retain intact water responses comparable to those of control animals ($n = 4$ for *Trpm5*$^{-/-}$ and *Trpm5*$^{+/-}$). (**b**) Application of amiloride (50 μM) completely blocked sodium responses while it did not exert significant effects on water responses ($n = 4$; $P = 0.2$ for water – amiloride versus water + amiloride). (**c**) Silencing acid-sensing TRCs (*Pkd2l1*^{TeNT} mice) eliminated both water responses and acid responses, while control animals (*TeNT*) show normal responses to both ($n = 5$ for saliva in control, $n = 6$ for the rest; $P = 0.0022$ for water). Values are means ± s.e.m. Diagrams in the bottom panel show the type of TRCs silenced in each experiment.

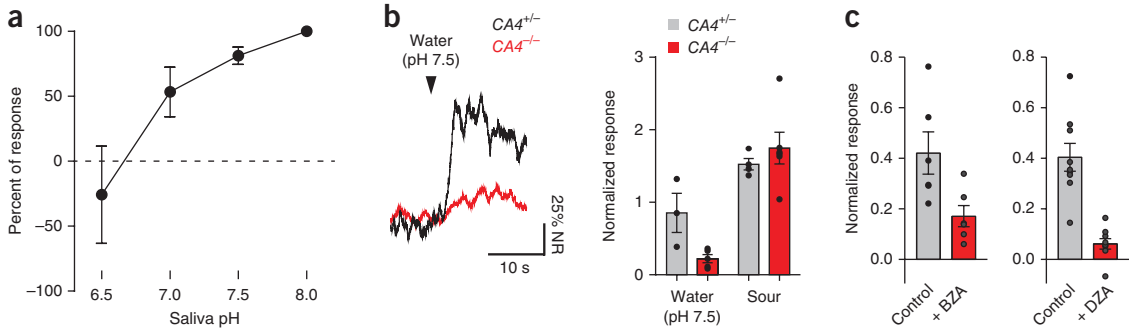

Figure 3 Carbonic anhydrases mediate taste responses to water. (**a**) Water responses are sensitive to salivary pH. All responses are normalized to the responses at pH 8 ($n = 4$; $P = 0.0392$ for pH 6.5 versus pH 7.5, two-tailed paired t-test). Saliva pH in healthy animals is normally neutral to basic[43]. (**b**) Representative traces from $CA4^{+/-}$ and $CA4^{-/-}$ mice showing responses to neutral water (adjusted to pH 7.5, left). $CA4^{-/-}$ mice have severely reduced responses to water ($n = 5$ for $CA4^{-/-}$, $n = 3$ for $CA4^{+/-}$; $P = 0.0357$, two-tailed Mann-Whitney U-test). NR, normalized response. (**c**) Water responses before and after incubation with the carbonic anhydrase inhibitors benzolamide (BZA) or dorzolamide (DZA). Both drugs reduced water responses ($n = 6$; $P = 0.0159$ for benzolamide, $n = 9$; $P = 0.0007$ for dorzolamide, two-tailed paired t-test). Values are means ± s.e.m.

Under dehydration, animals selectively drink water over other fluids (for example, oils). If the observed responses are the basis of water detection, we expect the responses to be highly selective for aqueous solutions. As hypothesized, application of nonaqueous silicone oil to the tongue did not evoke any nerve responses compared to water, indicating that the responses require aqueous medium in the oral cavity (**Fig. 1d**).

We next sought to identify the cellular substrate mediating water-induced taste responses. Previous studies have identified genetic markers that specifically label TRCs encoding individual taste qualities[11,18,30]. Using these genetic handles, we examined whether taste responses to water is independent of the previously described five basic tastes. Transgenic animals lacking TRPM5, a key transduction channel for umami, sweet and bitter[30], were unable to detect these

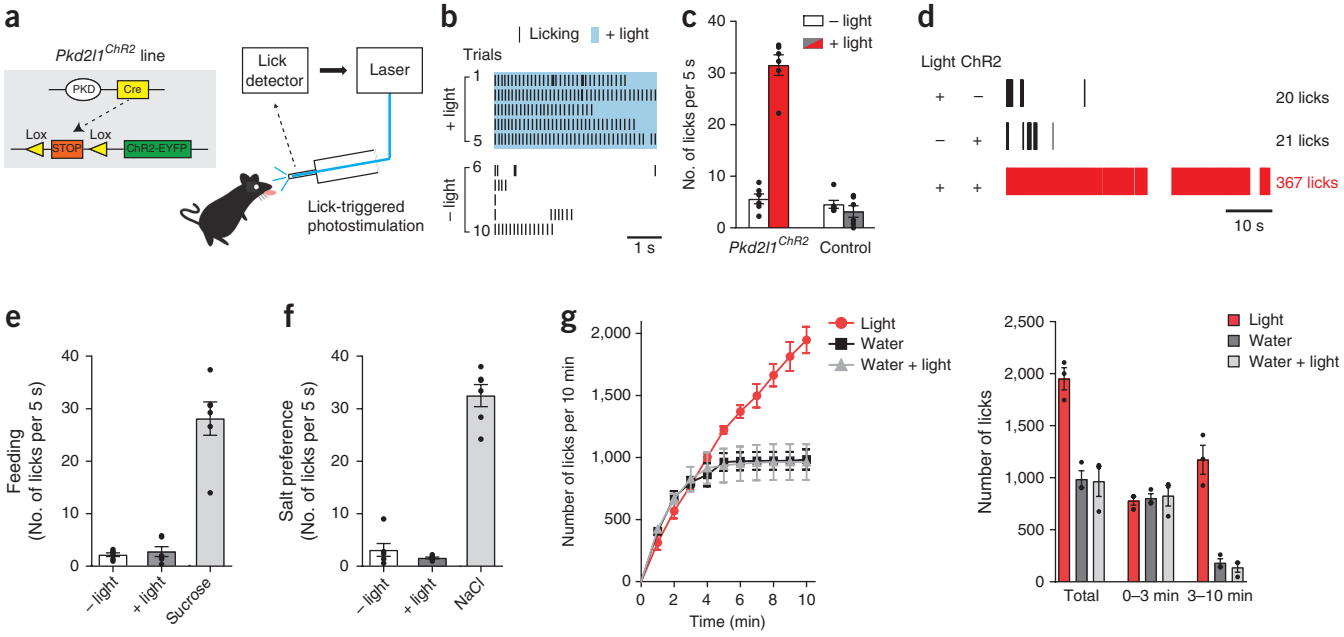

Figure 4 Stimulation of acid-sensing TRCs drives drinking responses. (**a**) Transgenic mice expressing ChR2 in PKD2L1-expressing TRCs ($Pkd2l1^{ChR2}$) were subjected to a close-loop self-stimulation experiment in which each lick induces a 1-s laser pulse though an optic fiber placed in an empty water spout. (**b**) Photostimulation of PKD2L1-expressing TRCs induced robust drinking responses without water (trials 1–5: blue shading). In the absence of light, the same water-deprived animal did not show consistent licking (trials 6–10). Each black bar indicates a lick event. (**c**) Quantification of light-dependent lick events. Licks were counted during a 5-s window. The number of licks was averaged across trials. The panel shows $Pkd2l1^{ChR2}$ mice ($n = 6$, red bar) and *Pkd2l1-Cre* control mice ($n = 6$, black bar) with photostimulation; white bars indicate the number of licks without light ($P = 0.0022$; two-tailed Mann-Whitney U-test). (**d**) Photostimulation induced continuous drinking responses. Shown are plots representative of at least 3 mice illustrating the drinking responses toward light in thirsty control (top) and $Pkd2l1^{ChR2}$ animals without (middle) or with light (bottom) during a 1-min session. Total number of licks is shown at right. (**e**,**f**) Food-deprived (**e**, $n = 6$) or salt-depleted (**f**, $n = 6$) $Pkd2l1^{ChR2}$ mice did not exhibit appetitive behavior toward light. NaCl or sucrose solutions (300 mM each) were used as control stimuli. (**g**) Photostimulation does not satiate animals. Shown are cumulative number of licks from $Pkd2l1^{ChR2}$ animals during 10-min behavioral sessions (left). Either water, light, or water + light was given during a session. Light stimulation induced significantly more total number of licks ($n = 3$, $P = 0.0145$; water versus light, two-tailed paired t-test) and licks during minutes 3–10 ($P = 0.0213$; water versus light, two-tailed paired t-test). Values are means ± s.e.m.

three taste modalities (**Fig. 2a**). In these animals, nerve responses to water were unaffected and indistinguishable from those in control animals (**Fig. 2a** and **Supplementary Fig. 2a**). Similarly, blocking the sodium taste sensor, ENaC, by its cognate antagonist (amiloride[31]) entirely suppressed sodium-evoked responses, but had no significant effect on nerve responses to water (**Fig. 2b**). Finally, we examined the involvement of acid-sensing TRCs by genetically silencing their synaptic machinery. To achieve this goal, we used transgenic animals in which tetanus toxin subunit was targeted to PKD2L1-positive cells by crossing Cre-dependent tetanus toxin (*TeNT*) and *Pkd2l1-Cre* transgenic lines[32] to yield *Pkd2l1*^{TeNT} mice. Surprisingly, disrupting synaptic transmission from acid-sensing TRCs resulted in a total loss of water responses (**Fig. 2c**) without affecting other taste qualities except acid (**Supplementary Fig. 2b**). Taken together, our data reveal the acid-sensing taste pathway as the cellular substrate underlying taste responses to water in addition to acids.

What is the mechanisms by which water activates TRCs? Given a function of PKD2L1-expressing TRCs as acid sensors (**Fig. 2c**), one possibility is that the water (saliva washout) stimulus may be converted to a local pH change, leading to the activation of this population. Consistent with this possibility, changing pH environment by washout of buffer with water significantly activated taste nerves (**Fig. 1b**). In fact, the amplitude of water responses was highly sensitive to saliva pH (**Fig. 3a**): excessive protons in saliva strongly suppressed water responses, indicating an important role of pH for the responses.

Carbonic anhydrase 4 (CA4) is a membrane-bound enzyme expressed by acid-sensing TRCs[32] and reversibly catalyzes the conversion of CO_2 and water into bicarbonate and protons. We hypothesized that washout of bicarbonate from saliva drives this reaction, leading to an increase in local proton production. If this is true, pharmacological blockade or knockout of CA4 should affect water-induced taste responses. In fact, mice lacking CA4 (encoded by *Car4*) exhibited significant and selective reduction in their water responses, although minor residual responses remained (**Fig. 3b** and **Supplementary Fig. 3a**). Similarly, CA blockers markedly suppressed water responses without affecting other taste responses (**Fig. 3c** and **Supplementary Fig. 3b**). These results suggest that carbonic anhydrases (mainly CA4) are the principal detectors that translate water stimuli into the local pH drop (**Supplementary Fig. 3c**). This model predicts that responses to water should have slower kinetics than responses to acids because it requires an additional step to activate the cells. Our analysis indicate that this is the case; activation dynamics of taste nerves by water were slower than those by citric acid (**Supplementary Fig. 3d**), supporting our idea that carbonic anhydrase-mediated local pH change is a major mechanism underlying water responses in TRCs.

Although our electrophysiological data showed that water specifically activates acid-sensing TRCs, it was still unclear whether these responses contribute to the detection of water. To address this question, we used an optogenetic strategy by engineering animals expressing channelrhodopsin[33] (ChR2) in PKD2L1-expressing TRCs (*Pkd2l1*^{ChR2}, **Fig. 4a**). Photostimulating the tongue with blue light in *Pkd2l1*^{ChR2} animals induced time-locked nerve responses, confirming functional ChR2 expression (**Supplementary Fig. 4a**). We reasoned that if we could successfully create an artificial water cue by photostimulation, thirsty animals should 'drink' light. To test this possibility, we set up a behavioral model in which animals have free access to an empty bottle attached to an optic fiber that provides touch-based feedback photostimulation (**Fig. 4a**). After a 2-d water-restriction regime, *Pkd2l1*^{ChR2} mice exhibited vigorous drinking responses toward light even in the absence of actual water (**Fig. 4b**,**c**). This behavior was light-intensity dependent (**Supplementary Fig. 4b**) and was observed

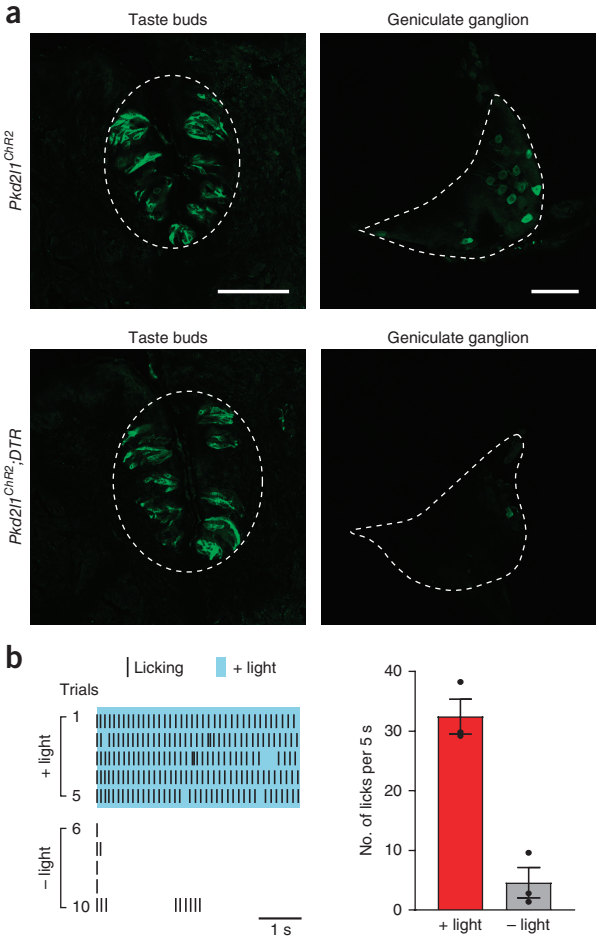

Figure 5 Genetic ablation of ChR2-positive geniculate neurons by diphtheria toxin. Since geniculate neurons project their dendrites to TRCs, the ChR2-expressing geniculate population may be activated by photostimulation of the tongue. We excluded this possibility by toxin-mediated cell ablation of this population. (**a**) Representative immunostaining of taste buds (left) and geniculate ganglia (right) from *Pkd2l1*^{ChR2} and diphtheria toxin-treated animals expressing diphtheria toxin receptor in the background of *Pkd2l1*^{ChR2} (*Pkd2l1*^{ChR2}*;DTR*). After 3–4 weeks of recovery, ChR2-EYFP-expressing cells regenerated in taste buds, but not in geniculate ganglia. Scale bars, 100 µm. (**b**) Left, light-induced licking responses in a toxin-treated *Pkd2l1*^{ChR2}*;DTR* animal using the same behavioral model described in **Figure 3b**. Photostimulation of the tongue induced robust drinking (trials 1–5: blue shading) after ablation of ChR2-positive geniculate neurons. Each black bar indicates a lick event. Right, quantification of drinking responses during 5 s ($n = 3$, $P = 0.026$, two-tailed paired-*t*-test). Values are means ± s.e.m.

both in a 5-s brief access test (**Fig. 4c**) and in a 1-min continuous test (**Fig. 4d** and **Supplementary Videos 1** and **2**). In contrast, neither control animals lacking ChR2 expression nor *Pkd2l1*^{ChR2} mice without photostimulation showed this behavior (**Fig. 4d**).

If activation of acid-sensing TRCs indeed provides a cue of water, then animals should be attracted to light only when they are thirsty. Thus, we explored the effect of photostimulation on various appetites such as sugar consumption in hungry animals (**Fig. 4e**) and salt appetite in sodium-depleted animals (**Fig. 4f**). As predicted, animals exhibited no behavioral attraction toward light under hungry and salt-craving conditions (**Fig. 4e**,**f**). These results substantiate the acid-sensing TRC population as a cellular substrate for external water detection.

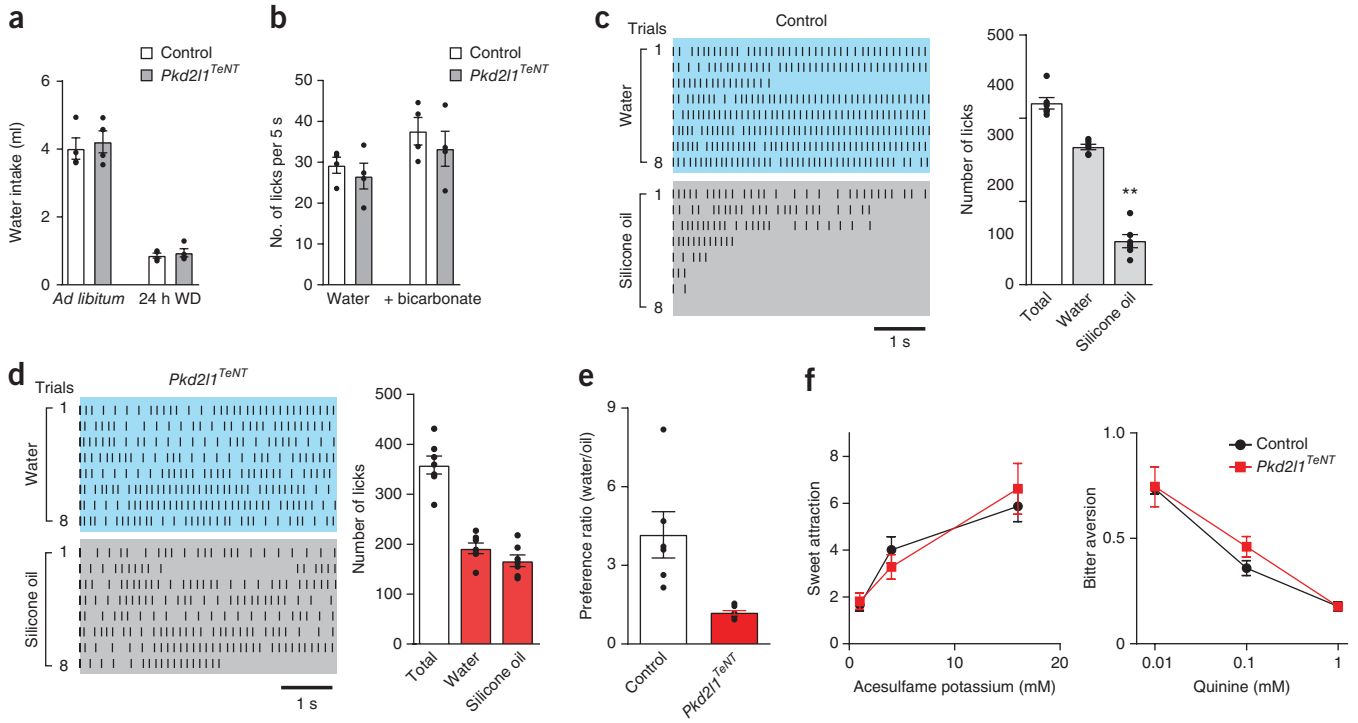

Figure 6 Water-induced taste signals provide a cue for fluid discrimination. Silencing acid-sensing taste pathway disrupts proper fluid choice. (**a**) *Ad libitum* and water deprivation (WD)-induced water intake in *Pkd2l1*^{TeNT} and control (*TeNT*) mice. Both genotypes consumed similar amount of water during 24 h for the *ad libitum* groups and during 15 min for the WD groups ($n = 4$ for each genotype). (**b**) In a two-bottle choice assay, water-deprived mice (23 h) exhibited no preference toward water and bicarbonate water (25 mM), which does not elicit taste responses to water ($n = 4$). (**c**) In a brief taste preference assay, water-deprived control mice (*TeNT*) showed strong preference toward water over silicone oil. Left, representative licking plots for water and silicone oil; fluids were presented for 8 times each. Each black bar indicates a lick event. The number of total licks as well as licks to each fluid was quantified ($n = 6$, $P < 0.0001$ for water versus silicone oil; two-tailed paired *t*-test). (**d**) In contrast, *Pkd2l1*^{TeNT} mice did not show preference for water and consumed a similar amount of silicone oil ($n = 7$). (**e**) Preference between water and oil was quantified as a ratio ($n = 7$ for *Pkd2l1*^{TeNT} and $n = 6$ for control; $P = 0.0012$, two-tailed Mann-Whitney *U*-test). (**f**) Both control and *Pkd2l1*^{TeNT} mice exhibited dose-dependent attraction to sweet (acesulfame potassium) and aversion to bitter (quinine), indicating that *Pkd2l1*^{TeNT} mice retain normal taste discrimination ($n = 4$ for *Pkd2l1*^{TeNT} and $n = 6$ for control). Data are show as a preference ratio to water. Values are means ± s.e.m.

We next asked whether the activation of acid-sensing TRCs also encodes satiation of water drinking. Thirsty animals normally drink to satiety within few minutes after water becomes available (**Fig. 4g**). Remarkably, water-deprived *Pkd2l1*^{ChR2} animals showed continuous and unimpeded licking toward light during entire behavioral sessions for 10 min (**Fig. 4g**). However, if water was present, animals stopped drinking after satiation even with photostimulation (**Fig. 4g**). Together, these results clearly demonstrate that activation of acid-sensing TRCs is sufficient to drive drinking, but does not evoke satiation with water.

In addition to the expression in taste buds, we noticed that ChR2 was ectopically expressed in a small number of geniculate neurons, secondary taste neurons that innervate taste buds (**Supplementary Fig. 5**). To eliminate the possibility that these neurons are involved in light-induced drinking responses, we expressed Cre-dependent diphtheria toxin receptor in the background of *Pkd2l1*^{ChR2} (*Pkd2l1*^{ChR2}*;DTR*, where *DTR* is derived from simian *Hbegf*) and ablated the entire population of PKD2L1-expressing cells by injection of diphtheria toxin (**Fig. 5a**). As TRCs but not secondary neurons regenerate over time, we were able to eliminate the contribution of ChR2-positive geniculate neurons. After regeneration of TRCs, we confirmed that *Pkd2l1*^{ChR2}*;DTR* mice still showed drinking response toward light, demonstrating that the behavior is driven by the activity of TRCs (**Fig. 5b**).

We next determined the contribution of taste signals to water drinking behavior. External water is detected through multiple orosensory systems including taste, temperature and tactile signals[34]. Even without taste signals (for example, in *Pkd2l1*^{TeNT} mice), animals showed normal spontaneous as well as thirst-induce drinking (**Fig. 6a**). Moreover, animals showed no preference regardless of bicarbonate concentration (**Fig. 6b**). These data suggest that this taste pathway is not required for water consumption. Instead, we wondered whether the taste pathway may help discriminate water from other nonaqueous liquids. To test this idea, *Pkd2l1*^{TeNT} and control animals were water-deprived and then given a choice between water and low-viscosity silicone oil in a brief taste preference test. As expected, the control group showed strong preference to water over silicone oil (**Fig. 6a**,**c** and **Supplementary Fig. 6a**,**b**). In contrast, *Pkd2l1*^{TeNT} animals failed to show preference between these two fluids and consumed both silicone oil and water (**Fig. 6b**,**c**). In the two-bottle assay, *Pkd2l1*^{TeNT} animals consumed more silicone oil but less water compared to the control group, partly because they drank both fluids and quickly became full. We observed a similar effect on mineral oil, another tasteless fluid (**Supplementary Fig. 6c**), although animals quickly learned to avoid using nontaste cues such as consistency. *Pkd2l1*^{TeNT} mice showed normal attraction to sweet and aversion to bitter compounds (**Fig. 6f**), indicating that they retain taste discrimination ability. Thus, signals via the acid-sensing taste pathway contribute to an appropriate fluid choice, but not to drinking action *per se*.

Previous studies have shown that acids activate PKD2L1-expressing TRCs, and eliminating these cells abolishes acid-evoked taste

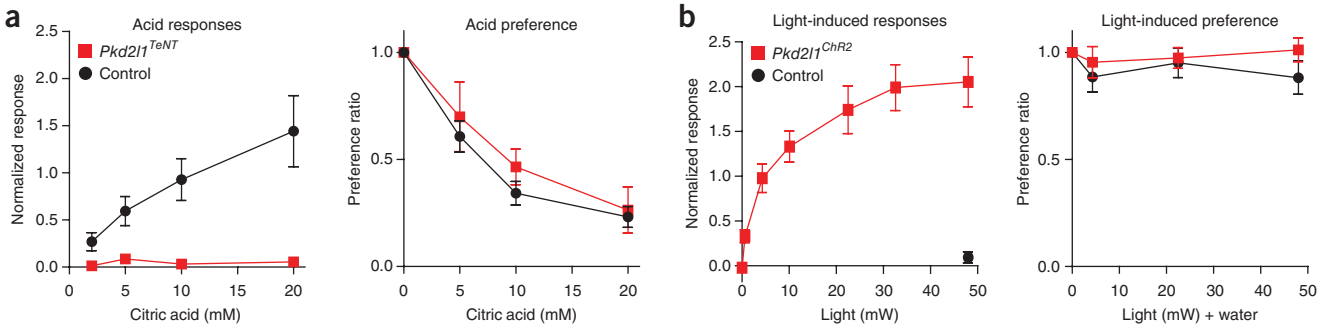

Figure 7 The acid-sensing taste pathway is not essential for sour aversion. (**a**) Normalized taste nerve responses to citric acid in *Pkd2l1*^{TeNT} and *TeNT* control animals (left). Control animals ($n = 6$) showed dose-dependent nerve responses to citric acid while *Pkd2l1*^{TeNT} ($n = 4$) mice showed no response ($P = 0.0095$, *Pkd2l1*^{TeNT} versus control at 20 mM citric acid). However, *Pkd2l1*^{TeNT} and control animals showed similar levels of aversion toward citric acid ($n = 6$ for control and $n = 4$ for *Pkd2l1*^{TeNT} mice), indicating that *Pkd2l1*-expressing TRCs are not necessary for aversive behavior to sourness. (**b**) Photostimulation of *Pkd2l1*-expressing TRCs fails to induce aversion. Left, chorda tympani nerve responses to different intensities of light in *Pkd2l1*^{ChR2} and Ai32 littermate controls with only the reporter ($n = 3$ for 0.04–32.6 mW for *Pkd2l1*^{ChR2} mice, $n = 4$ for the rest; $P = 0.0286$, *Pkd2l1*^{ChR2} versus control at 48 mW). Right, photostimulation of PKD2L1-expressing TRCs did not change preference toward water. Mice were given a bottle containing water with an optic fiber for stimulation. Control and *Pkd2l1*^{ChR2} mice showed undisturbed drinking behavior regardless of light intensity ($n = 7$ for control and $n = 6$ for *Pkd2l1*^{ChR2} mice). Statistical significance was analyzed with two-tailed Mann-Whitney *U*-test. Values are means ± s.e.m.

nerve responses[18,35]. Based on these findings, the PKD2L1-expressing TRC population has been suggested to mediate sour taste and associated aversive behavior. However, our results show that these TRCs also mediate water detection that drives appetitive drinking under thirst. To address this conundrum, we investigated whether PKD2L1-expressing TRCs are responsible for behavioral aversion to acid. Control animals with intact acid taste sensors exhibit robust aversion to citric acid, an acidic tastant (**Fig. 7a**). In contrast, we observed similar levels of aversion in *Pkd2l1*^{TeNT} animals while the taste nerve responses to acid were eliminated (**Fig. 7a**). These data indicate that the taste pathway is minimally required for behavioral aversion to acids. Conversely, optogenetic stimulation of the same population with ChR2 in *Pkd2l1*^{ChR2} animals triggered robust dose-dependent taste nerve firing (**Fig. 7b**). These mice, however, showed no obvious aversion toward water in the presence of light at any intensity (**Fig. 7b**). While PKD2L1-expressing TRCs function as acid sensors, our loss-of-function and gain-of-function experiments strongly suggest that additional, nontaste pathways mediate the aversive aspect of sour taste.

DISCUSSION

Peripheral water detection represents an important basis of fluid regulation in the body. Since Zotterman first described taste responses to water in frogs several decades ago[24], accumulating evidence supports such a finding in various vertebrate species[24–26,36]. These studies implicated specific water detection machinery in the vertebrate taste system. Here we used *in vivo* electrophysiology to elucidate mechanisms underlying water responses in the taste system. We found that washout of saliva with water activates the acid-sensing taste pathway through PKD2L1-expressing TRCs. Furthermore, our data imply that the removal of bicarbonate in saliva leads to local pH change through the activity of carbonic anhydrases expressed in PKD2L1-expressing TRCs. Because over 99% by volume of saliva is water, it seems logical that the taste system has a mechanism to detect the dilution of ions as a signal of incoming water, rather than sensing water itself. Together, these studies provide insights into the cellular and molecular basis of water detection at the periphery.

While stimulation of PKD2L1-expressing TRCs alone can drive appetitive drinking, our loss-of-function studies in the same population showed no defect in water consumption (**Fig. 6**). These observations suggest that nontaste signals are sufficient to drive normal levels of water detection and consumption even without taste signals. Although the physiological importance of PKD2L1-mediated water detection remains unknown, the sensory redundancy with respect to water may help maintain body fluid balance even if one sensory pathway fails.

It has been previously demonstrated[32,37] that PKD2L1-expressing TRCs are activated by acids, salts, and CO_2, and we now find that they also respond to water. These stimuli are ultimately converted to protons that activate the population. Physiologically, these TRCs are proton sensors mediating acid responses, as shown previously[35,38]. What taste information do these TRCs encode? In this study, we found that optogenetic activation of this taste pathway by light triggered appetitive licking responses in thirsty animals. Conversely, functional manipulations of PKD2L1-expressing TRCs had little effect on acid-induced aversive behavior. These results argue that this taste pathway detects noxious substances and hence may contribute to aversion when combined with other sensory signals[32,37]. However, our study shows that the activation of this pathway by itself may not directly encode negative valence. Besides the taste system, other sensory pathways including the trigeminal system also contribute to orosensation[34]. Because various noxious chemicals are known to activate both taste and trigeminal nerves[39], it is conceivable that behavioral aversion to acids, salt and CO_2 are partly mediated by nontaste sensory pathways[40]. At the perceptual level, there are two potential models to explain attractive behavior induced by the acid taste pathway: first, this population may encode nonaversive sour perception (flavor) and animals may use it to detect external fluids. Second, these TRCs may evoke a taste perception distinct from sour (for example, water or ionic) and sour perception may be transmitted through concurrent activation of taste and nontaste pathways. Although we cannot distinguish these possibilities, identifying nontaste sour signals and further psychophysical studies will enable us to address how taste perception of water and sour is integrated in the brain.

Notably, behavioral attraction by photostimulation of the acid-sensing taste pathway was induced only when animals were dehydrated, but not food or salt depleted. This internal-state dependency is a distinctive characteristic as compared to basic tastes such as sweet or bitter that are innately linked to positive and negative values[41].

While the mechanisms of this valence change are unknown, hypothalamic and reward circuits are likely involved in this process[5,42]. Future studies with neural manipulations of peripheral water pathway and central thirst circuits should help address how appetite and the hedonic value of water are encoded in the brain.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

D.Z. and Y.O. conceived the research program. D.Z. and Y.O. designed and carried out the experiments and analyzed data. G.W. maintained and provided CA4 knockout animals. D.Z. analyzed data and, together with Y.O., wrote the paper. Y.O. supervised the entire work.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Animals. All procedures were carried out in accordance with US National Institutes of Health (NIH) guidelines for the care and use of laboratory animals and received approval from the Caltech Institutional Animal Care and Use Committees (IACUC; approval #1694-14G). *C57BL/6ByJ* (B6, stock number 00664), *Ai32* (stock number 012569) and *Rosa26iDTR* (stock number 08040) mice were obtained from the Jackson Laboratory. Transgenic lines used were *Trmp5* knockout (ref. 30), *Pkd2l1-Cre* (ref. 18) and *Rosa26-flox-TeNT* (ref. 44), as described previously. For optogenetic experiments, *Pkd2l1*^{ChR2} mice were generated by crossing *Pkd2l1-Cre* and *Ai32* lines. *Rosa26-flox-DTR* was crossed with the *Pkd2l1*^{ChR2} line to generate *Pkd2l1*^{ChR2}*;DTR* mice for cell-ablation experiments. Mice used for data collection were both males and females, at least 6 weeks of age. Animals were group-housed in a temperature-controlled environment with a 13-h light and 11-h dark cycle and *ad libitum* access to food and water unless otherwise noted. To minimize the use of animals, we used the same group of animals for multiple behavioral tests.

Nerve recordings. Mice were anesthetized with pentobarbital (100 mg/kg) and placed in a head-fixation apparatus. Body temperature was monitored and regulated using a closed-loop heating system. Chorda tympani taste nerve recordings were performed as previously described[12,37]. Briefly, animals were given a tracheotomy to prevent suffocation and the right branch of the chorda tympani nerve was exposed. A high-impedance tungsten electrode was hooked onto the nerve and a drop of halocarbon oil was dropped inside the surgical cavity. Stimuli were delivered using a pressurized perfusion system (AutoMate Scientific) to keep a constant flow rate. Stimuli used were 60 mM NaCl (salt), 10 mM citric acid (sour), 8 mM acesulfame potassium (sweet), 0.1 mM cycloheximide (bitter), 50 mM monopotassium glutamate plus 1 mM inosine monophosphate (umami), 5-centistoke silicone oil (Aldrich) and mineral oil (Aldrich). Deionized water was either filtered (Elaga, PURELAB flex) or purchased (ultra-purified water, Invitrogen 10977-015). All solutions were used at room temperature. Nerve responses during the 20-s tastant stimulation were integrated and analyzed. Responses in each recording session were normalized to 8 mM acesulfame potassium (**Figs. 1**, **2b**,**c**,**e**,**f** and **5**), 10 mM citric acid (**Fig. 2a**), and to 25 mM $KHCO_3$ (**Fig. 1c**). For **Figure 1b**, individual responses were normalized to the average 8 mM acesulfame potassium responses across entire sessions. The artificial saliva composition was as follows: 4 mM NaCl, 10 mM KCl, 6 mM $KHCO_3$, 6 mM $NaHCO_3$, 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.24 mM K_2HPO_4, 0.24 mM KH_2PO_4. pH was held between 7.4 and 7.6. For **Figure 3a**, the pH of artificial saliva was adjusted between 6 and 8. To stabilize pH of water in CA4 experiments (**Fig. 3b**), we added 2 mM $KHCO_3$ and adjusted pH to 7.5. Each tastant stimulation was followed by intervals at least for 40 s. Pharmacological experiments were conducted as follows: 50 μM amiloride was dissolved into tastant stimulus solutions and delivered via the pressurized perfusion system. Tastant solutions containing amiloride were presented for 20 s, preceded and followed by 5-s incubation periods with saliva containing the same concentration of amiloride. The oral cavity was incubated with a membrane-impermeable carbonic anhydrase blocker, benzolamide (650 μM), or a membrane-permeable blocker, dorzolamide (0.5%), in water for 7 min before washing out with saliva as described previously[32].

Analysis of activation kinetics of taste nerves. Activation kinetics of nerve responses was analyzed using similar methods as previously described[45]. The time points of 25% and 75% of maximum amplitude in each response were determined using Matlab, and the rise time was calculated as the difference between these two points (**Supplementary Fig. 3d**). The ratio of water to sour rising-phase slopes was calculated as the slope of the line connecting the points at 25% to 75% of the peak amplitude of the response.

Taste preference assays. Animals were tested in a custom gustometer to measure taste preference as previously described[12]. Solutions were presented to animals for 60 s per trial. Each behavioral session comprised 10 to 30 trials, depending on the number of tastants tested. Each stimulus was presented at least 5 times in one session. Presentations automatically terminated 5 s after the first lick. The number of licks in each of these 5-s windows were counted and then averaged across the session. Each animal was tested for up to 3 sessions with the same taste repertoire. We freshly prepared solutions for each behavioral experiment to minimize contamination by other sensory cues such as odors. Prior to all behavioral experiments, mice were water restricted for 23–46 h. For experiments that extended more than 23 h, animals were provided with 0.5 mL of water after 23 h. For sucrose appetite assays, animals had no access to food for the 23 h before the experiments (**Fig. 4e**). For salt appetite assays (**Fig. 4f**), mice were injected with furosemide as previously described[37] and were kept for 23 h on a low-sodium diet (Envigo 90228) with free access to water. For sucrose and salt appetite experiments, we used 300 mM sucrose and NaCl. Before testing with photostimulation, animals were pretrained to drink these solutions.

For drinking assays for silicone oil and water (**Fig. 6** and **Supplementary Fig. 6**), animals were pretrained to drink water and oil in a gustometer before testing. In 5-s brief access test (**Fig. 6**), oil and water were presented in the same behavioral session at least five times each. In the 5-min assay (**Supplementary Fig. 6**), individual fluids were tested on separate dates; water tests were normally followed by silicone oil tests because some animals were euthanized after the silicone oil assays because of dehydration. We noted that both control and *Pkd2l1*^{TeNT} animals preferred water in a long-term *ad libitum* drinking assay.

For quantifying spontaneous and thirst-driven drinking, animals were individually placed in their home cages and water intake was monitored for 24 h (*ad libitum*) or for 15 min (after water restriction for 23 h).

Cell ablation by injection of diphtheria toxin. *Pkd2l1*^{ChR2}*;DTR* mice were given an intramuscular injection of diphtheria toxin fragment A (20 μg/kg BW per day, Sigma D0564) for 2 consecutive days. Expression of ChR2 on the tongue was monitored before, during and after injections to assess amount of ablation. After the 2-d injection regime, mice were housed 3–4 weeks before being used for behavioral experiments to allow regeneration of TRCs.

Optogenetic stimulation. Photostimulation of the tongue was performed using a gustometer as described above. Animals were subjected to a brief access taste preference assay as follows: (i) two empty bottles with and without an optic fiber or (ii) solutions with and without an optic fiber. Blue laser pulses (430–490 nm, Shanghai Lasers and Optics Century Co.) were delivered through an optic fiber (1 mm diameter, ThorLabs) using a pulse generator (World Precision Instruments). Every lick triggered a laser pulse of 1 s duration in a closed-loop manner. The laser power was kept at 48 mW (measured at the tip) unless otherwise noted.

Photostimulation of taste nerves. The same surgery for nerve recording was performed as outlined above. For optogenetic stimulation, the tip of an optic fiber was placed a few millimeters from the tongue while nerve responses from *Pkd2l1*^{ChR} were recorded. Trains of light pulses of 20 s duration were flashed onto the tongue. Each 20-s stimulation window was followed by a 40-s interval. Light trains were delivered at 8 Hz, with each pulse of 40 ms duration. The frequency and duration was determined based on the licking behavior of mice in our behavioral assays.

Histology. Animals were euthanized with CO_2 followed by cervical dislocation and perfused with PBS followed by 4% PFA. Tongues were removed and kept in 4% PFA for 12 h followed by 30% sucrose in PBS for overnight. Frozen sections of 20 μm thickness were prepared on slides and were postfixed with 4% PFA for 15 min. Then the samples were incubated with blocking buffer (10% donkey serum, 0.2% Triton-X) for 2 h before incubation with primary antibodies. Primary antibodies used were goat anti-CA4 (R&D Systems, 1:500, AF2414), anti-GFP (Abcam, 1:1000, ab6673) and rabbit anti-PLC-β2 (Santa Cruz Biotechnologies, 1:500, sc-206). Rabbit PKD2L1 antibody[19] (1:500) was a generous gift from H. Matsunami at Duke University. Secondary antibodies were donkey anti-goat Cy3 (Jackson ImmunoResearch, 1:500, 705-165-147) and donkey anti-rabbit Alexa-488 (Jackson ImmunoResearch, 1:500, 711-545-152), donkey anti-chicken Alexa-488 (Jackson ImmunoResearch, 1:500, 703-545-155). For each representative image, the experiments were successfully repeated at least three times.

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Statistical analysis. Depending on the experimental design, we used either two-tailed Mann-Whitney *U*-test or paired *t*-test. Values indicate the number of animals used for the experiments. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications[5,12]. Data distribution was assumed to be normal, but this was not formally tested. No randomization was used for the data collection. Data collection and analysis were not performed blind to the conditions of the experiments, although key behavioral experiments such as **Figure 4** were repeated by other laboratory members in a blind fashion. Data points were excluded from the analysis if surgery was unsuccessful.

Data and code availability. The data that support the findings of this study are provided in source data for **Figures 1**, **3**, **4**, **6** and **7**, and code is available from the corresponding author upon reasonable request.

A **Supplementary Methods Checklist** is available

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