Salt taste in mammals can trigger two divergent behavioural responses. In general, concentrated saline solutions elicit robust behavioural aversion, whereas low concentrations of NaCl are typically attractive, particularly after sodium depletion\(^1\). Notably, the attractive salt pathway is selectively responsive to sodium and inhibited by amiloride, whereas the aversive one functions as a non-selective detector for a wide range of salts\(^2\). Because amiloride is a potent inhibitor of the epithelial sodium channel (ENaC), ENaC has been proposed to function as a component of the salt-taste receptor system\(^3\). Previously, we showed that four of the five basic taste qualities—sweet, sour, bitter and umami—are mediated by separate taste-receptor cells (TRCs) each tuned to a single taste substrate for all five basic taste qualities, and validate the essential role of ENaC for sodium taste in mice.

Sodium is the major cation of extracellular fluids and an essential component of every fluid compartment in the body. It is therefore not surprising that animals have evolved dedicated salt-sensing systems, including prominent detectors in the taste system\(^1\). These salt-sensitive receptors are crucial for the acceptance of low concentrations of sodium (for example, to satisfy the ‘salt appetite’\(^1\)) while simultaneously serving as a warning mechanism against hyper-salinity\(^1\), thus helping to maintain ion and water homeostasis. For humans, the ‘taste for salt’ also has direct bearing on excessive Na\(^+\) consumption, which is believed to be a significant dietary risk factor in hypertension, particularly in the developed world\(^1\). In mice, the low-concentration, and behaviourally ‘attractive’ salt-taste pathway has three salient properties: it is activated at NaCl concentrations as low as 10 mM, it is highly selective for sodium versus other cations, and it is blocked by lingual application of the ion-channel inhibitor amiloride\(^1\). The high-concentration (aversive) pathway, conversely, begins to be significant only at concentrations greater than 150 mM NaCl; it is non-selective for sodium (that is, other salts are equally effective), and it is amiloride-insensitive.

To explore the cellular basis for the taste of NaCl (that is, determine whether the distinct physiological and behavioural responses are mediated by the same or separate TRCs), we developed a new preparation that allows functional imaging of TRCs in response to salt stimulation. In essence, TRCs in fungiform papillae were loaded with the calcium-sensitive dye calcium green\(^2\) in vivo, and then were stimulated and imaged, ex vivo, with a regime that either preferentially activated the low-concentration pathway (100 mM NaCl), or activated both the high- and low-concentration pathways (500 mM NaCl). To separate the contribution of each of the two salt-sensing systems at high-stimulation concentrations, we examined the salt responses in the presence and absence of 10 μM amiloride (Fig. 1). Receptor cells that are only activated by high concentrations of salt also respond to a wide range of non-sodium salts (for example, from KCl to N-methyl-D-glucamine (NMDG)-Cl; Fig. 1 and Supplementary Fig. 1), and their activity is unaffected by the presence of amiloride (Fig. 1b, c). In contrast, low-concentrations of NaCl activate a completely separate population of TRCs; these cells do not respond to non-sodium salts (Fig. 1a, c, see also Supplementary Fig. 1), and their responses are blocked by amiloride (Fig. 1c). These results demonstrate the presence of two anatomically distinct salt-sensing systems, and accordingly suggest that the appetitive and aversive behaviours are mediated by non-overlapping populations of TRCs. As the TRCs activated by low concentrations of NaCl are highly selective for sodium salts, we consider them to be the dedicated sodium-sensing system and thus are the subject of this study.

Because amiloride is an inhibitor of the epithelial sodium channel (ENaC), ENaC has been proposed to be a potential component of the salt-taste receptor system\(^1\). The ENaC channel is made up of three subunits (α, β and γ), and has an important role in regulating trans-epithelial transport of Na\(^+\) in a wide range of tissues, including kidneys, airway cells of the lung, epithelial skin cells, and the ducts of salivary and sweat glands. Although the knockout of any ENaC subunit is sufficient to completely abolish ENaC function\(^1\), conventional ENaC knockouts die within a few days of birth\(^2\), precluding their use in physiological and behavioural studies of taste.

To examine the role of ENaCs in the taste system, we used a floxed ENaC conditional knockout strategy (Scnn1a\(^{lox/lox}\)\(^{\text{Flox}}\)). In essence, we generated animals in which ENaC function was selectively eliminated in all differentiated TRCs by using the cytokeratin19 gene—a marker for all mature taste cells (see Supplementary Fig. 2) to drive the expression of Cre-recombinase in the taste system. To investigate the taste responses of the conditional ENaC knockout mice, we recorded tastant-induced action potentials from nerves innervating the taste cells of the tongue; this physiological assay monitors the activity of the taste system at the periphery and provides a reliable measure of TRC function\(^3\). In wild-type mice, NaCl elicits a dose-dependent increase in action potentials in the chorda tympani nerve, which is blocked in the ENaC conditional knockout mice.

Salt taste elicits two divergent behavioural responses: an appetitive pathway that is specific to NaCl, and an aversive pathway that responds to a wide range of salts, including non-sodium salts. Recently, we showed that TRCs express the ENaC subunits, and validate the essential role of ENaC for sodium taste in mice. Sodium is the major cation of extracellular fluids and an essential component of every fluid compartment in the body. It is therefore not surprising that animals have evolved dedicated salt-sensing systems, including prominent detectors in the taste system. These salt-sensitive receptors are crucial for the acceptance of low concentrations of sodium (for example, to satisfy the ‘salt appetite’) while simultaneously serving as a warning mechanism against hyper-salinity, thus helping to maintain ion and water homeostasis. For humans, the ‘taste for salt’ also has direct bearing on excessive Na\(^+\) consumption, which is believed to be a significant dietary risk factor in hypertension, particularly in the developed world. In mice, the low-concentration, and behaviourally ‘attractive’ salt-taste pathway has three salient properties: it is activated at NaCl concentrations as low as 10 mM, it is highly selective for sodium versus other cations, and it is blocked by lingual application of the ion-channel inhibitor amiloride. The high-concentration (aversive) pathway, conversely, begins to be significant only at concentrations greater than 150 mM NaCl; it is non-selective for sodium (that is, other salts are equally effective), and it is amiloride-insensitive.

To explore the cellular basis for the taste of NaCl (that is, determine whether the distinct physiological and behavioural responses are mediated by the same or separate TRCs), we developed a new preparation that allows functional imaging of TRCs in response to salt stimulation. In essence, TRCs in fungiform papillae were loaded with the calcium-sensitive dye calcium green in vivo, and then were stimulated and imaged, ex vivo, with a regime that either preferentially activated the low-concentration pathway (100 mM NaCl), or activated both the high- and low-concentration pathways (500 mM NaCl). To separate the contribution of each of the two salt-sensing systems at high-stimulation concentrations, we examined the salt responses in the presence and absence of 10 μM amiloride (Fig. 1). Receptor cells that are only activated by high concentrations of salt also respond to a wide range of non-sodium salts (for example, from KCl to N-methyl-D-glucamine (NMDG)-Cl; Fig. 1 and Supplementary Fig. 1), and their activity is unaffected by the presence of amiloride (Fig. 1b, c). In contrast, low-concentrations of NaCl activate a completely separate population of TRCs; these cells do not respond to non-sodium salts (Fig. 1a, c, see also Supplementary Fig. 1), and their responses are blocked by amiloride (Fig. 1c). These results demonstrate the presence of two anatomically distinct salt-sensing systems, and accordingly suggest that the appetitive and aversive behaviours are mediated by non-overlapping populations of TRCs. As the TRCs activated by low concentrations of NaCl are highly selective for sodium salts, we consider them to be the dedicated sodium-sensing system and thus are the subject of this study.

Because amiloride is an inhibitor of the epithelial sodium channel (ENaC), ENaC has been proposed to be a potential component of the salt-taste receptor system. The ENaC channel is made up of three subunits (α, β and γ), and has an important role in regulating trans-epithelial transport of Na\(^+\) in a wide range of tissues, including kidneys, airway cells of the lung, epithelial skin cells, and the ducts of salivary and sweat glands. Although the knockout of any ENaC subunit is sufficient to completely abolish ENaC function, conventional ENaC knockouts die within a few days of birth, precluding their use in physiological and behavioural studies of taste.

To examine the role of ENaCs in the taste system, we used a floxed ENaC conditional knockout strategy (Scnn1a\(^{lox/lox}\)\(^{\text{Flox}}\)). In essence, we generated animals in which ENaC function was selectively eliminated in all differentiated TRCs by using the cytokeratin19 gene—a marker for all mature taste cells (see Supplementary Fig. 2) to drive the expression of Cre-recombinase in the taste system. To investigate the taste responses of the conditional ENaC knockout mice, we recorded tastant-induced action potentials from nerves innervating the taste cells of the tongue; this physiological assay monitors the activity of the taste system at the periphery and provides a reliable measure of TRC function. In wild-type mice, NaCl elicits a dose-dependent increase in action potentials in the chorda tympani nerve.
stimuli (Fig. 2d and Supplementary Fig. 3). In contrast, ENaC to four of the five basic taste qualities: sweet, bitter, umami and sour
consume low to moderate concentrations of NaCl (Fig. 2). As would be expected if ENaC was the sodium sensor, it should mediate all attraction to salt, and consequently, the knockout mice should have a total loss of behavioural attraction to NaCl. Indeed, ENaC knockout mice show a complete loss of the responses to low concentrations of NaCl (Fig. 2). Loss of ENaC activity in the taste system does not affect responses to four of the five basic taste qualities: sweet, bitter, umami and sour stimuli (Fig. 2d and Supplementary Fig. 3). In contrast, ENaC knockouts show a complete loss of the responses to low concentrations of NaCl (Fig. 2). As would be expected if ENaC was the sodium sensor, these animals are also missing all amiloride-sensitivity in their NaCl responses (Fig. 2). Importantly, the knockout mice retain all responses to non-sodium salts (Fig. 2d and Supplementary Fig. 3). These results demonstrate that taste responses to salts are mediated by genetically separable components.

Animals ranging from simple invertebrates to mammals readily consume low to moderate concentrations of NaCl, and actively seek it under conditions of salt deprivation. Therefore, we carried out behavioural tests of salt consumption to examine the taste behaviour of the ENaC conditional knockout animals both under conditions of salt depletion (to test attraction) and under water deprivation (to test aversion). We reasoned that if ENaC encodes the principal sodium taste sensor, it should mediate all attraction to salt, and consequently, the knockout mice should have a total loss of behavioural attraction to NaCl. Indeed, ENaC knockout mice show no significant attraction to salt, even under conditions in which control animals have an extraordinary appetite for sodium (Fig. 3a). In contrast, the aversive responses to high concentrations of NaCl (and KCl) are unaltered in the same knockout animals (Fig. 3b, c). Notably, behavioural responses to sweet, sour, umami and bitter

**Figure 1** | **Two classes of TRCs mediate distinct salt taste responses.**

Fungiform taste buds loaded with the activity sensor calcium green respond with high selectivity and specificity to different concentrations of salt. a. A unique subset of TRCs ([low]) respond to low concentrations of sodium chloride (100 mM) as well as higher concentrations (500 mM) but not to other salts (KCl). Shown below the imaging data are individual traces from four different TRCs depicting the kinetic and amplitude changes in intracellular calcium levels after salt stimulation; calcium changes were

![Figure 1](image_url)

pseudo-coloured as depicted. b. A different population of TRCs ([high]) are activated only at increased NaCl concentrations (500 mM) and are also stimulated by KCl; shown are individual traces for three different TRCs. c. Amiloride selectively blocks [low] responses but has no effect on [high] responses; shown are individual traces for four different TRCs; the duration of tastant application is denoted by black bars. See Supplementary Fig. 1 for a diagram of the preparation, quantifications and responses to other salts.

with a physiological response threshold of approximately 10 mM (Fig. 2). Loss of ENaC activity in the taste system does not affect responses to four of the five basic taste qualities: sweet, bitter, umami and sour tastants are indistinguishable from control animals (Fig. 3d). These results validate ENaC as the mammalian taste receptor responsible for behavioural acceptance of (and attraction to) NaCl.

Our previous studies have shown that sweet, bitter, umami and sour tastes are mediated by independent populations of TRCs, each tuned to a single taste modality. If this labelled-line logic of taste coding at the periphery extends to all five basic taste modalities, then sodium taste should also be mediated by a unique population of TRCs. Thus, we examined whether the amiloride-sensitive salt-sensing cells indeed define a sub-population of TRCs separate from sweet, bitter, sour and umami TRCs. We engineered mice expressing Cre-recombinase under the control of the ENaCα gene, and then crossed them to a floxed green fluorescent protein (GFP) reporter line (Z/EG). To validate the fidelity of Cre expression in ENaCα-expressing cells, we analysed progeny from four independent Cre-driver founders and confirmed proper GFP reporter expression in the airway cells of the lung as well as in the kidney cortical collecting duct cells and distal convoluted tubules—well-characterized sites of ENaCα expression (see Supplementary Fig. 4).

Co-labelling with the sweet/umami/bitter TRC marker, TrpM5 (ref. 19), demonstrated that ENaCα-expressing cells are distinct from sweet-, bitter- or umami-TRGs (Fig. 4a and Supplementary Fig. 6). In fungiform and palate taste buds co-localization with a sour cell marker, Car4 (ref. 27), showed the presence of two populations of TRGs: one exhibiting co-expression of ENaCα and Car4 (Fig. 4b), and importantly, a second one expressing ENaCα but not sour, sweet,
bitter or umami markers (referred to as 'ENaC-alone' cells; Fig. 4b, c). We hypothesized that the ENaC-alone cells are the bona-fide sodium taste sensors, and that the expression of ENaCα in sour cells may just be a consequence (that is, non-functional) of a common lineage between the cells mediating ionic tastes. Thus, we carried out further studies. First, we generated animals lacking ENaCα solely in Car4-expressing cells by using a sour-cell Cre driver to excise the conditional ENaCα knockout allele. As expected, these mice show wild-type responses to sweet, bitter, umami and sour stimuli. Notably, they have normal salt responses that are indistinguishable from wild-type controls (Supplementary Fig. 5), thus demonstrating that the ENaCα expression in the sour cells is in fact not required for salt taste. In a complementary study, we also generated mice entirely lacking sour-sensing cells\(^\text{15}\); these animals show a total loss of sour sensing, yet they maintain normal salt responses (Supplementary Fig. 5). Most critically, we directly imaged salt and sour responses using our new peeled epithelium preparation. Indeed, there is total segregation of the cells responding to salt (low and high concentrations) versus those responding to acid stimulation (that is, sour cells never respond to salt stimuli\(^\text{15}\); see Supplementary Fig. 1). Taken together, these results substantiate the functional and anatomical segregation of sodium-sensing TRCs, and prove that all five basic taste modalities are mediated by separate and dedicated receptor cells at the periphery.

An unusual feature of the physiology of sodium taste in mice has been the observation that the back of the tongue (circumvallate papillae) contains no sodium-selective (amiloride-sensitive) responses\(^\text{10,22,28}\), highlighting a strong topographic segregation (front to back) in salt taste (see later). With the identity of the amiloride-sensitive salt taste receptor at hand, we reasoned that it should now be possible to explore the molecular basis of the absence of sodium sensing at the back of the tongue. ENaC channels are composed of...
three essential subunits (α, β and γ), thus we anticipated that this Na⁺- and amiloride-insensitivity could be easily explained if the functional ENaC heterotrimeric channel was not found in the circumvallate papillae. Indeed, our results show that at the back of the tongue, ENaCα and ENaCβ subunits are found in completely non-overlapping populations of TRCs (Supplementary Fig. 6). Therefore, amiloride-insensitivity at the back of the tongue is due to the lack of functional ENaC channels.

In this study we have shown that ENaC, first proposed to have a role in salt taste more than 25 years ago, functions as the sodium taste receptor. We also demonstrated that sodium taste is mediated by a dedicated population of TRCs separate from those mediating sweet, umami, bitter and sour taste. Notably, the taste of sodium and non-sodium salts are detected by genetically, pharmacologically and physiologically distinguishable TRCs. The availability of two channels (and cellular pathways) for salt-sensing endows animals with the ability to distinguish sodium-containing salts from other salts; this affords mammals with a powerful mechanism to select food sources containing adequate sodium but at the same time to avoid ingesting excessive amounts of salt.

The presence of salt shakers on dinner tables around the world attests to the appetite role of salt taste in the human diet. Indeed, salt has been a food additive shared by humans for thousands of years, with empires from the Roman (for salary) to the British (for taxes) valuing it as a precious commodity. Does ENaC function in human salt taste? Physiological recordings in non-human primates have clearly demonstrated an amiloride-sensitive component in taste responses to salt stimuli19,30. However, psychophysical experiments in humans remain inconclusive7, with some reports of amiloride altering salt taste2,12, and several failing to substantiate a significant effect for amiloride in the perception of saltiness (reviewed in ref. 7).

Given the molecular similarities between mice and humans in all other taste modalities16, a ‘human-specific’ molecular mechanism for salt taste would be surprising. Perhaps more likely, the contribution of ENaC to human salt taste may be masked as a result of experience, exposure to salt, and diet. Future experiments studying people subjected to controlled salt intake may help clarify the role, if any, of ENaC in human taste.

METHODS SUMMARY

Transgenic animals and mouse strains. ENaCα-IRES-Cre, ENaCβ-IRES-ITA and cytochromeP450-IRES-Cre are bacterial artificial chromosome (BAC)-transgenics engineered to express Cre-recombinase or the tetracycline-dependent transactivator (tTA) by inserting an IRES-Cre or IRES-ITA transgene 3′ to the Scnn1a, Scnn1b or Krt19 stop codon. Other strains have been described previously24,25.

Calcium imaging. Fungiform TRCs were pre-loaded in vivo with Calcium Green-1 dextran 3kDa (Invitrogen) by electroporation single taste buds. After 24–36 h, taste epithelium was enzymatically peeled11,18 and placed on a recording chamber with the apical side of TRCs facing up (Supplementary Fig. 1). Taste stimuli were delivered in artificial saliva by focal application. Changes in intracellular calcium ([Ca²⁺]i) were imaged using a 5-Live confocal microscope (Zeiss) and the relative change in fluorescence (ΔF/F) from individual TRCs analysed and pseudo-coloured as described previously26.

Nerve recording, behavioural and localization studies. All procedures were as described previously5,9,14,25,27.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 4 November 2009; accepted 5 January 2010.

Published online 27 January 2010.

1. Contreras, R. J. in Neural Mechanisms in Taste (ed. Cagan, R. H.) 119–145 (CRC, 1989).
2. Duncan, C. J. Salt preferences of birds and mammals. Physiol. Zool. 35, 120–132 (1962).
3. Lindemann, B. Receptors and transduction in taste. Nature 413, 219–225 (2001).
4. Beaughamp, K. G., Bertino, M., Burke, D. & Engelman, K. Experimental sodium depletion and salt taste in normal human volunteers. Am. J. Clin. Nutr. 51, 881–889 (1990).
5. Mueller, K. L. et al. The receptors and coding logic for bitter taste. Nature 434, 225–229 (2005).
6. Eylam, S. & Spector, A. C. Taste discrimination between NaCl and KCl is disrupted by amiloride in inbred mice with amiloride-insensitive chorda tympani nerves. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288, R1361–R1368 (2005).
7. Halpern, B. P. Amiloride and vertebrate gustatory responses to NaCl. Neurosci. Biobehav. Rev. 23, 5–47 (1998).
8. Heck, G. L., Miersom, S. & DeSimone, J. A. Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. Science 223, 403–405 (1984).
9. Hettlinger, T. P. & Frank, M. E. Specificity of amiloride inhibition of hamster taste responses. Brain Res. 513, 24–34 (1990).
10. Doolin, R. E. & Gilbertson, T. A. Distribution and characterization of functional amiloride-sensitive sodium channels in rat tongue. J. Gen. Physiol. 107, 545–554 (1996).
11. Kretz, O., Barbry, P., Bock, R. & Lindemann, B. Differential expression of RNA and protein of the three pore-forming subunits of the amiloride-sensitive epithelial sodium channel in taste buds of the rat. J. Histochem. Cytochem. 47, 51–64 (1999).
12. Schifman, S. S., Lockhead, E. & Maes, F. W. Amiloride reduces the taste intensity of Na⁺ and Li⁺ salts and sweeteners. Proc. Natl Acad. Sci. USA 80, 6136–6140 (1983).
13. Vandebuch, A., Clapp, T. R. & Kinnamom, S. C. Amiloride-sensitive channels in type I fungiform taste cells in mouse. BMC Neurosci. 9, 1 (2008).
14. Yoshida, R. et al. NaCl responsive taste cells in the mouse fungiform taste buds. Neuroscience 159, 795–803 (2009).
15. Huang, A. L. et al. The cells and logic for mammalian sour taste detection. Nature 442, 934–938 (2006).
16. Yarmolinsky, D. A., Zaker, C. S. & Ryba, N. J. Common sense about taste: from mammals to insects. Cell 139, 234–244 (2009).
17. Zhang, Y. et al. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. Cell 112, 293–301 (2003).
18. Zhao, G. Q. et al. The receptors for mammalian sweet and umami taste. Cell 115, 255–266 (2003).
19. Canessa, C. M. et al. Amiloride-sensitive epithelial Na⁺ channel is made of three homologous subunits. Nature 367, 463–467 (1994).
20. Himmler, E. & Beermann, F. Scnn1b sodium channel gene family in genetically engineered mice. J. Am. Soc. Nephrol. 11, S529–S534 (2000).
21. Strazzullo, P., D’Elia, L., Kandala, N. B. & Cappuccio, F. P. Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. BMJ. doi:10.1136/bmj.b4567 (2009).
22. Nimmoriya, Y. Reinnervation of cross-regenerated gustatory nerve fibers into amiloride-sensitive and amiloride-insensitive taste receptor cells. Proc. Natl Acad. Sci. USA 95, 5347–5350 (1998).
23. Oka, Y. et al. Odorant receptor map in the mouse olfactory bulb: in vivo sensitivity and specificity of receptor-defined glomeruli. Neuron 52, 857–869 (2006).
24. Himmler, E., Merillat, A. M., Rubera, I., Rossier, B. C. & Beermann, F. Conditional gene targeting of the Scnn1a (eNaC) gene locus. Genesis 32, 169–172 (2002).
Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank W. Guo and A. Becker for generation and maintenance of mouse lines, and K. Scott and members of our laboratories for valuable comments. This research was supported in part by the intramural research program of the NIH, NIDCR (N.J.P.R.). C.S.Z. is an investigator of the Howard Hughes Medical Institute.

Author Contributions J.C. designed the study, carried out electrophysiological and expression studies, analysed data and wrote the paper; C.K. designed and carried out behavioural experiments and analysed expression in engineered and knockout mice; Y.O. designed and carried out calcium imaging experiments and analysed data; D.A.Y. carried out molecular studies and helped write the paper; E.H. provided essential reagents; N.J.P.R. and C.S.Z. designed the study, analysed data and wrote the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/nature. Correspondence and requests for materials should be addressed to C.S.Z. (cz2195@columbia.edu).
METHODS

Transgenic animals and mouse strains. The PKD2L1-IRES-Cre, ROSA-DTA, Z/EG and ENaC conditional knockout (Scnn1a\(^{floxed}\)) strains have been described previously\(^{15,24}\). ENaCz-IREs-Cre, ENaC\(z\)-IRES-tTA and cytokeratin19-IREs-Cre are BAC-transgenics engineered to express Cre-recombinase or the tetracycline dependent transactivator (TTA) by inserting an IRES-Cre or IRES-tTA transgene 3' to the Scnn1a, Scnnib or Krt19 stop codon. The ENaC\(z\)-IRES-tTA transgenic mice also carried a TetO-sapphire (modified GFP) reporter in the BAC-transgene. Four independent lines of ENaCz-IREs-Cre expressed the Cre transgene appropriately in kidney and lung and labelled equivalent populations of taste cells (Supplementary Figs 4 and 6). Similarly, three founder lines expressing ENaC\(z\)-IRES-tTA showed equivalent TTA-expression in lung, kidney and taste tissue. Mice were inter-crossed as described in the text to generate appropriate tissue. Mice were inter-crossed as described in the text to generate appropriate

Calcium imaging. Fungiform TRCs were pre-loaded in vivo with calcium green-1 dextran 3kD (Invitrogen) by electroporating single taste buds in the tongue of anaesthetized mice using a 3.5 \(\mu\)A, 50 pulses s\(^{-1}\) \(\times\) 16 cycles regime. On average, 8–9 taste buds were loaded per animal. After 24–36 h of recovery, tongues were removed and the taste epithelium enzymatically peeled as described previously\(^{11}\). The epithelium was then placed on a recording chamber with the apical side of taste buds facing up (Supplementary Fig. 1), ensuring that the integrity and polarity of the epithelium was maintained. The apical surface of the preparation was bathed in artificial saliva at a constant flow rate of 4.5 ml min\(^{-1}\), and taste stimuli were delivered by focal application using a custom made dispensing pipette (800 \(\mu\)m diameter). Tastant application was for 1 s, with a minimum of 10 s of artificial saliva between stimuli. Changes in \([Ca^{2+}]_{i}\) were imaged using a 5-Live confocal microscope (Zeiss) using an ×40 C-Apochromat 1.20W objective; images were captured at 4 Hz, and A/F from individual TRCs analysed and pseudo-coloured as described previously\(^{15}\).

Nerve recordings. Lingual stimulation and recording procedures were performed as previously described\(^{17,25}\). All data analyses used the integrated response over a 25-s period immediately after the application of the stimulus. Tastants used for nerve recordings were: 3–30 mM acesulphameK (sweet); 1–100 mM monopotassium glutamate plus 1.0 mM inosine monophosphate (umami); 1–10 mM quinine hydrochloride (bitter); 1–50 mM citric acid (sour). The responses to 50 mM citric acid were used to normalize responses to each experimental series in control and ENaCz-KO (Fig. 2). To compute the amiloride-sensitive salt component (Fig. 2b), the stimulation regime involved sequential applications of NaCl solutions first without and then with amiloride (in the same experimental series). The amiloride-insensitive component was defined as the response in the presence of amiloride (Fig. 2c). The fraction of the response inhibited by amiloride was defined as the amiloride-sensitive component (amiloride-sensitive component = response without amiloride – response with amiloride; Fig. 2b). Responses in experiments involving PKD2L1-IRES-CreRosa-DTA (PKD2L1-DTA) and PKD2L1-IRES-CreENaCz flox/flox (PKD2L1-ENaC-KO) were normalized to responses obtained with 30 mM acesulphameK (Supplementary Fig. 5). NaCl solutions used in dose-response studies for measuring the amiloride-insensitive sodium responses (Fig. 2c) included 10 \(\mu\)M amiloride. Differences between knockout and control responses were analysed for statistical significance using an unpaired, two-tailed Student’s \(t\)-test and 95% confidence limits.

Behavioural assays. Behavioural assays used a custom-made gustometer to measure immediate lick responses as described previously\(^{17,18}\). For salt-attraction assays, mice were injected with furosemide (50 mg kg\(^{-1}\)) and were placed on a low sodium diet with unrestricted water for 16–20 h to deplete sodium before testing\(^{1}\). For salt-aversion assays, mice were water deprived for 24 h before testing\(^{1,18}\). Control tastants were 32 mM acesulphameK (sweet), 100 mM monosodium glutamate plus 1 mM inosine monophosphate and 0.1 mM amiloride (umami), 1 mM quinine sulphate (bitter), and 150 mM citric acid (sour). Differences between knockout and control responses were analysed for statistical significance using an unpaired, two-tailed Student’s \(t\)-test and 95% confidence limits; for Supplementary Fig. 5b a one-way analysis of variance (ANOVA) with Newman–Keuls posterior test was used to compare data sets.

Immunohistochemistry and cell labelling. Immunostaining, whole-mount imaging (GFP) and in situ hybridization were performed as described previously\(^{17,18}\). Animals were perfused with 4% paraformaldehyde and tissue was post-fixed for 6–48 h to allow localization of GFP. Images were obtained using a Leica SP2 TSC or a Zeiss 510 LSM meta confocal microscope. Anti-TrpM5 and anti-Car4 antibodies were as described previously\(^{17,27}\). The illustration in Fig. 4c was composed by converting the red and green channels in Fig. 4b to greyscale and overlaying with the ENaC-alone (green only) cells using Adobe Photoshop.