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Involvement of multiple taste receptors in umami taste: analysis of gustatory nerve responses in metabotropic glutamate receptor 4 knockout mice

Keiko Yasumatsu[1,2], Tomohiro Manabe[1,3], Ryusuke Yoshida[1], Ken Iwatsuki[4], Hisayuki Uneyama[5], Ichiro Takahashi[3] and Yuzo Ninomiya[1,2]

[1]*Section of Oral Neuroscience, Graduate School of Dental Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*
[2]*Division of Sensory Physiology, Research and Development Center for Taste and Odor Sensing, Kyushu University, Fukuoka, Japan*
[3]*Section of Orthodontics and Dentofacial Orthopedics, Graduate School of Dental Sciences, Kyushu University, Fukuoka, Japan*
[4]*Department of Nutritional Science and Food Safety, Faculty of Applied Biosciences, Tokyo University of Agriculture, Tokyo, Japan*
[5]*Institute for Innovation, Ajinomoto Co., Inc., Kawasaki, Japan*

Key points

- The taste receptor T1R1 + T1R3 heterodimer and metabotropic glutamate receptors (mGluR) may function as umami taste receptors.
- Here, we used mGluR4 knockout (mGluR4-KO) mice and examined the function of mGluR4 in peripheral taste responses of mice.
- The mGluR4-KO mice showed reduced responses to glutamate and L-AP4 (mGluR4 agonist) in the chorda tympani and glossopharyngeal nerves without affecting responses to other taste stimuli.
- Residual glutamate responses in mGluR4-KO mice were suppressed by gurmarin (T1R3 blocker) and AIDA (group I mGluR antagonist).
- The present study not only provided functional evidence for the involvement of mGluR4 in umami taste responses, but also suggested contributions of T1R1 + T1R3 and mGluR1 receptors in glutamate responses.

Abstract Umami taste is elicited by L-glutamate and some other amino acids and is thought to be initiated by G-protein-coupled receptors. Proposed umami receptors include heterodimers of taste receptor type 1, members 1 and 3 (T1R1 + T1R3), and metabotropic glutamate receptors 1 and 4 (mGluR1 and mGluR4). Accumulated evidences support the involvement of T1R1 + T1R3 in umami responses in mice. However, little is known about the *in vivo* function of mGluR in umami taste. Here, we examined taste responses of the chorda tympani (CT) and the glossopharyngeal (GL) nerves in wild-type mice and mice genetically lacking mGluR4 (mGluR4-KO). Our results indicated that compared to wild-type mice, mGluR4-KO mice showed significantly smaller gustatory nerve responses to glutamate and L-(+)-2-amino-4-phosphonobutyrate (an agonist for group III mGluR) in both the CT and GL nerves without affecting responses to other taste stimuli. Residual glutamate responses in mGluR4-KO mice were not affected by (*RS*)-alpha-cyclopropyl-4-phosphonophenylglycine (an antagonist for group III mGluR), but were suppressed by gurmarin (a T1R3 blocker) in the CT and (*RS*)-1-aminoindan-1,5-dicarboxylic acid (an antagonist for group I mGluR) in the CT and GL nerve. In wild-type mice, both quisqualic acid (an agonist for group I mGluR) and L-(+)-2-amino-4-phosphonobutyrate elicited gustatory nerve responses and these responses were suppressed by addition of (*RS*)-1-aminoindan-1,5-dicarboxylic acid and (*RS*)-alpha-cyclopropyl-4-phosphonophenylglycine, respectively. Collectively, the present study provided functional evidences for the involvement of mGluR4 in umami taste responses in mice.

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The results also suggest that T1R1 + T1R3 and mGluR1 are involved in umami taste responses in mice. Thus, umami taste would be mediated by multiple receptors.

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Corresponding author K. Yasumatsu and Y. Ninomiya: Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Email: yasuma@dent.kyushu-u.ac.jp or yuninom@dent.kyushu-u.ac.jp

Abbreviations AIDA, (*RS*)-1-aminoindan-1,5-dicarboxylic acid; AMPA, α-amino-3-hydroxy-5-methl-4-isoxazole propionic acid; CPPG, (*RS*)-alpha-cyclopropyl-4-phosphonophenylglycine; CT, chorda tympani; CV, circumvallate papillae; D-AP5, D-(−)-2-amino-5-phosphonopentanoic acid; DW, distilled water; FP, fungiform papillae; GL, glossopharyngeal; Gur, gurmarin; IMP, inosine monophosphate; KO, knockout; L-AP4, L-(+)-2-amino-4-phosphonobutyrate; mGluR1 (mGluR4), metabotropic glutamate receptor type 1 (type 4); MPG, monopotassium glutamate; MSG, monosodium glutamate; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulphonamide; NMDA, *N*-methyl-D-aspartic acid; QHCl, quinine-HCl; Quis, quisqualic acid; T1R1 (T1R2 or T1R3), taste receptor family 1 member 1 (2 or 3); WT, wild-type.

Introduction

Umami taste is elicited by L-glutamate, other amino acids (e.g. L-aspartate), some peptides and certain ribonucleotides that are natural constituents of many protein-rich foods. A salient feature of umami taste in rodents and humans is the impressive potentiation by purine nucleotides (Yamaguchi, 1967). This umami synergism probably occurs at the umami taste receptor T1R1 + T1R3 (Zhang *et al.* 2008; Behrens *et al.* 2011). In heterologous expression systems, the human T1R1 + T1R3 heterodimer is activated by glutamate (Li *et al.* 2002), whereas the mouse T1R1 + T1R3 is activated by various amino acids (Nelson *et al.* 2002). In both the human and the mouse T1R1 + T1R3, occurrence of synergism between glutamate and inosine monophosphate (IMP) is demonstrated. Gene knockout (KO) model mice having a deletion of the amino-terminal extracellular domain but not the seven transmembrane helices of T1R1 and/or T1R3 showed that the chorda tympani (CT) nerve responses to glutamate and preference for glutamate were totally absent (Zhao *et al.* 2003). Thus, T1R1 + T1R3 is well known as an umami taste receptor in both humans and rodents. However, recent studies using another KO model mice genetically lacking T1R1 or T1R3 proteins suggest the existence of umami taste receptors other than T1R1 + T1R3 (Damak *et al.* 2003; Delay *et al.* 2006; Maruyama *et al.* 2006; Yasumatsu *et al.* 2012; Kusuhara *et al.* 2013). In both T1R1-KO and T1R3-KO mice, glutamate responses of taste cells and gustatory nerves were diminished but not abolished accompanied by alteration in behavioural responses.

Which receptor(s) do underlie residual umami responses in T1R1-KO and T1R3-KO mice? Several agonists for ionotropic glutamate receptors such as kainic acid, *N*-methyl-D-aspartic acid (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) induced no umami taste in humans (Kurihara & Kashiwayanagi, 2000), therefore it is unlikely that subtypes of ionotropic glutamate receptors function as umami taste receptors. In contrast, metabotropic glutamate receptors (mGluRs) and/or their variants are potential candidates for umami taste receptors other than T1R1 + T1R3. A taste-specific variant of mGluR4 (taste-mGluR4), which lacks most of the N-terminal extracellular domain, was identified in taste buds of circumvallate (CV) and foliate papillae in rats (Chaudhari *et al.* 1996). When expressed in Chinese hamster ovary cells, this receptor responded to glutamate and the group III mGluR agonist L-(+)-2-amino-4-phosphonobutyrate (L-AP4), although the affinity of taste-mGluR4 for glutamate and L-AP4 was more than 100-fold lower than that of brain-type receptors (Chaudhari *et al.* 1996, 2000, 2009; Yang *et al.* 1999). In addition, full-length mGluR1 and mGluR4 (Toyono *et al.* 2002, 2003) and a variant of mGluR1 (taste-mGluR1), which lacks much of the N-terminal extracellular domain (San Gabriel *et al.* 2005), are expressed in a subset of taste cells in rats. The taste-mGluR1 has more than 100-fold lower affinity for glutamate relative to the brain-type receptor (San Gabriel *et al.* 2005, 2009). However, little is known about the function and contributions of these mGluRs on umami taste sensitivity.

To address this issue we used wild-type (WT) and mGluR4-KO mice and recorded their whole nerve responses in the CT and the GL nerves to various taste stimuli, and agonists with and without antagonists for potential glutamate receptors (mGluR1, mGluR4). We show the relative contribution of mGluR4 in total umami taste information that occurred from the tongue, and the function of each umami receptor, including mGluR1 and T1R1 + T1R3, in the anterior and posterior parts of the tongue.

Methods

Ethical approval

All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the Committee for Laboratory Animal Care and Use and the local ethics committee at Kyushu University, Japan.

Animals

Subjects were adult male and female mGluR4-KO (Pekhletski *et al.* 1996) mice, which were back-crossed to C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) for at least five generations. Adult male and female C57BL/6J mice were also used as WT mice. All mice were maintained on a 12/12 h light/dark cycle and fed standard rodent chow. Animals were 8–20 weeks of age, ranging in weight from 20 to 35 g.

Gustatory nerve recording

Whole nerve responses to lingual application of tastants were recorded from the CT or the glossopharyngeal (GL) nerve as described previously (Ninomiya, 1998; Damak *et al.* 2003; Yasumatsu *et al.* 2003; Kusuhara *et al.* 2013). Mice were anaesthetized with an injection of sodium pentobarbital (40–50 mg kg^{-1} I.P.) and maintained at a surgical level of anaesthesia with supplemental injections of sodium pentobarbital (8–10 mg kg^{-1} I.P. every hour). Under pentobarbital anaesthesia, the trachea of each mouse was cannulated, and the mouse was then fixed in the supine position with a head holder to allow dissection of the CT or the GL nerve. The right CT nerve was dissected free from surrounding tissues after removal of the pterygoid muscle and cut at the point of its entry to the tympanic bulla. The right GL nerve from a different animal was exposed, dissected free from underlying tissues and cut near its entrance to the posterior lacerated foramen. The entire nerve was placed on the Ag-AgCl electrode. An indifferent electrode was placed in nearby tissue. Neural activities were amplified (K-1; Iyodenshikagaku, Nagoya, Japan), and monitored on an oscilloscope and audiomonitor. Whole nerve responses were integrated with a time constant of 1.0 s and recorded on a computer for later analysis using a PowerLab system (PowerLab/sp4; AD Instruments, Bella Vista, Australia).

For taste stimulation of fungiform papillae (FP), the anterior half of the tongue was enclosed in a flow chamber made of silicone rubber (Ninomiya & Funakoshi, 1981). For taste stimulation of the CV and foliate papillae, an incision was made on each side of the animal's face from the corner of the mouth to just above the angle of the jaw, and the papillae were exposed and their trenches opened by slight tension applied through a small suture sewn in the tip of the tongue. All chemicals were used at ∼24°C. Taste solutions were delivered to each part of the tongue by gravity flow for 30 s (CT) or 60 s (GL) at the same flow rate as the distilled water (DW) used for rinse (∼0.1 ml s^{-1}). We used a longer stimulation time for the GL nerve response recording, which is thought to be, unlike stimulation of fungiform taste buds located on the tongue surface, necessary for delivered test solutions to reach CV and foliate taste buds located on basal side walls of the deep and narrow trench formed by taste papillae and surrounding tissues. The tongue was washed with DW for an interval of 1 min between successive stimulation. Only responses from stable recordings were used for data analysis. At the end of the experiment, animals were killed by an overdose of anaesthetic.

Solutions

Test stimuli were 100 mM NH_4Cl, 10–1000 mM NaCl, 0.1–10 mM HCl, 10–1000 mM sucrose, 0.1–20 mM quinine HCl (QHCl) and 10–1000 mM monopotassium glutamate (MPG) with and without 0.5 mM IMP. These chemicals were dissolved in DW. Agonists and antagonists for glutamate receptors used were: 0.1 μM–3 mM quisqualic acid (Quis), 0.1 μM–3 mM L-AP4, 0.03–3 mM (*RS*)-1-aminoindan-1,5-dicarboxylic acid (AIDA), 0.03–3 mM (*RS*)-alpha-cyclopropyl-4-phosphonophenylglycine (CPPG), 3 mM D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5) and 3 mM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulphonamide (NBQX). Quis is an agonist for group I mGluRs (mGluR1 and 5) and AMPA receptors. L-AP4 is an agonist for group III mGluR (mGluR4, 6–8). AIDA is an antagonist for group I mGluR. CPPG is an antagonist for group III mGluR. D-AP5 is an antagonist for NMDA receptors. NBQX is an antagonist for both kainic acid receptors and AMPA receptors. These agonists and antagonists were dissolved in DW with KOH to adjust their pH to 7.0. Because previous studies demonstrated that taste mGluR have lower affinities than the corresponding brain type receptors (Chaudhari *et al.* 2000; San Gabriel *et al.* 2005) and taste responses to glutamate were inhibited by high concentrations of AIDA and CPPG (Nakashima *et al.* 2001; Eschle *et al.* 2009; Yasumatsu *et al.* 2012; Kusuhara *et al.* 2013), we also tested high concentration of antagonists. These antagonists are mixed with MPG to apply to the tongue. To neglect the potential effect of K^+ from KOH in these solutions, the same concentration of KCl was supplemented to the MPG solution. To block responses via T1R1 + T1R3 (Daly *et al.* 2013), the tongue was treated with 30 μg ml^{-1} (∼7 μM) gurmarin (Gur) dissolved in 5 mM phosphate buffer (pH 6.8) for 10 min in the same manner as described by Ninomiya & Imoto (1995). Reagents were purchased

Table 1. Repeated measures ANOVA results of concentration series of taste compounds in the CT and GL (mGluR4 knockout *vs.* wild-type mice; Figs 2 and 3)

Stimulus nerve	Genotype	Concentration	Genotype × concentration
HCl			
CT	$F_{1,40} = 0.039$, $P = 0.847$	$F_{4,40} = 83.103$, $P < 0.001$	$F_{4,40} = 0.151$, $P = 0.962$
GL	$F_{1,44} = 0.740$, $P = 0.408$	$F_{4,44} = 145.188$, $P < 0.001$	$F_{4,44} = 0.498$, $P = 0.737$
NaCl			
CT	$F_{1,52} = 1.642$, $P = 0.222$	$F_{4,52} = 279.147$, $P < 0.001$	$F_{4,52} = 1.196$, $P = 0.324$
GL	$F_{1,36} = 0.042$, $P = 0.843$	$F_{4,36} = 116.532$, $P < 0.001$	$F_{4,36} = 0.241$, $P = 0.913$
Sucrose			
CT	$F_{1,55} = 0.650$, $P = 0.437$	$F_{5,55} = 122.470$, $P < 0.001$	$F_{5,55} = 0.457$, $P = 0.806$
GL	$F_{1,40} = 0.192$, $P = 0.673$	$F_{5,40} = 2.580$, $P = 0.041$	$F_{5,40} = 0.128$, $P = 0.985$
QHCl			
CT	$F_{1,55} = 0.196$, $P = 0.667$	$F_{5,55} = 109.619$, $P < 0.001$	$F_{5,55} = 1.545$, $P = 0.191$
GL	$F_{1,36} = 0.884$, $P = 0.317$	$F_{4,36} = 46.748$, $P < 0.001$	$F_{4,36} = 0.213$, $P = 0.935$
MPG			
CT	$F_{1,48} = 7.893$, $P = 0.016$	$F_{4,48} = 45.477$, $P < 0.001$	$F_{4,48} = 0.247$, $P = 0.910$
GL	$F_{1,64} = 9.925$, $P = 0.006$	$F_{4,64} = 183.176$, $P < 0.001$	$F_{4,64} = 1.334$, $P = 0.267$
MPG + IMP			
CT	$F_{1,48} = 5.141$, $P = 0.043$	$F_{4,48} = 22.750$, $P < 0.001$	$F_{4,48} = 0.338$, $P = 0.851$
GL	$F_{1,56} = 12.743$, $P = 0.003$	$F_{4,56} = 158.369$, $P < 0.001$	$F_{4,56} = 1.349$, $P = 0.264$

Abbreviations: CT, chorda tympani; GL, glossopharyngeal; IMP, inosine monophosphate; MPG, monopotassium glutamate; QHCl, quinine HCl.

Table 2. Repeated measures ANOVA results for the effect of addition of IMP on concentration series of MPG in the CT and GL (MPG + IMP mixture *vs.* sum of MPG and IMP; Figs 2 and 3)

Genotype nerve	IMP	Concentration	IMP × concentration
WT			
CT	$F_{1,56} = 12.846$, $P = 0.003$	$F_{4,56} = 29.639$, $P < 0.001$	$F_{4,56} = 1.363$, $P = 0.258$
GL	$F_{1,60} = 0.127$, $P = 0.727$	$F_{4,60} = 130.869$, $P < 0.001$	$F_{4,60} = 0.613$, $P = 0.654$
mGluR4-KO			
CT	$F_{1,40} = 19.279$, $P = 0.001$	$F_{4,40} = 35.790$, $P < 0.001$	$F_{4,40} = 1.207$, $P = 0.323$
GL	$F_{1,60} = 0.051$, $P = 0.825$	$F_{4,60} = 301.835$, $P < 0.001$	$F_{4,60} = 1.987$, $P = 0.108$

Abbreviations: CT, chorda tympani; GL, glossopharyngeal; IMP, inosine monophosphate; KO, knockout; MPG, monopotassium glutamate; QHCl, quinine HCl; WT, wild-type. Differences between mixture of MPG with 0.5 mM IMP and the sum of each component were assessed by repeated measures ANOVA.

from Tocris Bioscience (Bristol, UK; L-AP4, Quis, AIDA, CPPG, D-AP5), Ajinomoto (Tokyo, Japan; IMP disodium salt), Sigma (St Louis, MO, USA; MPG) and Wako Pure Chemical Industries (Osaka, Japan; others).

Data analysis

In the analysis of whole nerve responses, integrated whole nerve response magnitudes were measured 5, 10, 15, 20 and 25 s (for the CT) and 5, 10, 20, 30 and 40 s (for the GL) after stimulus onset. These values were averaged and normalized to responses to 100 mM NH_4Cl to account for mouse-to-mouse variations in absolute responses. This relative response was used for statistical analysis (two-way ANOVA and *post-hoc* Fisher test, one-way ANOVA, and Student's unpaired *t* test or paired *t* test). Two-way ANOVA, one-way ANOVA and Student's *t* test were used to evaluate statistically the difference between genotype (mGluR4-KO and WT mice), treatments (with and without 0.5 mM IMP, Gur or glutamate receptor antagonists) or concentration. Calculations were performed using the statistical software package IBM SPSS Statistics (IBM, Armonk, NY, USA).

Results

Basic taste experiment

Both mGluR4-KO and WT mice showed robust CT nerve responses to NaCl, HCl, sucrose and QHCl in all concentrations tested (Figs 1 and 2). These CT

Table 3. Results of Student's *t* test for the occurrence of responses to agonists for glutamate receptors in the CT and GL of wild-type mice (Fig. 4)

Agonist	CT	GL
0.1 μM Quis	$t_{18} = 0.236$, $P = 0.816$	$t_{18} = 0.769$, $P = 0.452$
1 μM Quis	$t_{18} = 1.15$, $P = 0.265$	$t_{18} = 1.283$, $P = 0.216$
10 μM Quis	$t_{18} = 1.125$, $P = 0.233$	$t_{18} = 1.073$, $P = 0.297$
100 μM Quis	$t_{18} = 0.542$, $P = 0.594$	$t_{18} = 2.522$, $P = 0.021$
300 μM Quis	$t_{18} = 0.770$, $P = 0.451$	$t_{18} = 2.294$, $P = 0.034$
1 mM Quis	$t_{18} = 2.133$, $P = 0.047$	$t_{18} = 2.136$, $P = 0.047$
3 mM Quis	$t_{18} = 3.615$, $P = 0.002$	$t_{18} = 2.687$, $P = 0.015$
0.1 μM L-AP4	$t_{18} = 1.542$, $P = 0.140$	$t_{18} = 0.576$, $P = 0.572$
1 μM L-AP4	$t_{18} = 0.454$, $P = 0.655$	$t_{18} = 1.073$, $P = 0.297$
10 μM L-AP4	$t_{18} = 0.292$, $P = 0.773$	$t_{18} = 0.452$, $P = 0.657$
100 μM L-AP4	$t_{18} = 0.273$, $P = 0.788$	$t_{18} = 2.427$, $P = 0.026$
300 μM L-AP4	$t_{18} = 1.388$, $P = 0.182$	$t_{18} = 2.523$, $P = 0.021$
1 mM L-AP4	$t_{18} = 2.200$, $P = 0.041$	$t_{18} = 2.534$, $P = 0.021$
3 mM L-AP4	$t_{18} = 3.087$, $P = 0.006$	$t_{18} = 2.975$, $P = 0.008$

Abbreviations: L-AP4, L(+)-2-amino-4-phosphonobutyrate; CT, chorda tympani; GL, glossopharyngeal; Quis, quisquaric acid. Significant responses were detected based on the comparison of neural activities between before and after stimulation onset with each agonist. *t* test.

nerve responses were not significantly different between mGluR4-KO and WT mice by a repeated measures ANOVA test (effect of genotype: $P > 0.1$, Table 1). In marked contrast, CT nerve responses to MPG and MPG + IMP in mGluR4-KO mice were significantly smaller than those in WT mice (repeated measures ANOVA, a main effect of genotype; $F_{1,48} = 7.893$; $P = 0.016$ for MPG, $F_{1,48} = 5.141$; $P = 0.043$ for MPG + IMP, Fig. 2, Table 1). Synergism between MPG and IMP was observed in both WT and mGluR4-KO mice (repeated measures ANOVA, effect of IMP: $F_{1,56} = 12.846$; $P = 0.003$ for WT, $F_{1,40} = 19.279$; $P = 0.001$ for KO, Table 2). Similar results were observed in the GL nerves except for the occurrence of synergism (Figs 1 and 3; Tables 1 and 2). GL nerve responses to NaCl, HCl, sucrose and QHCl were not significantly different between mGluR4-KO and WT mice (repeated measures ANOVA, effect of genotype: $P > 0.1$, Table 1). In contrast, GL nerve responses to MPG and MPG + IMP in mGluR4-KO mice were significantly smaller than those in WT mice (repeated measures ANOVA, effect of genotype: $F_{1,64} = 9.925$; $P = 0.006$ for MPG, $F_{1,56} = 12.743$; $P = 0.003$ for MPG + IMP; Fig. 3 and Table 1). Synergism between MPG and IMP was not detected in the GL nerves of both WT and mGluR4-KO mice (repeated measures ANOVA, effect of IMP: $P > 0.1$, Table 2). These results indicate that mGluR4 is involved in the detection of umami compounds.

Agonist experiment

If mGluRs contribute to the detection of umami taste, their agonists would elicit gustatory nerve responses. In addition, their antagonists would at least in part inhibit umami taste responses. We first tested whether agonists for mGluR elicit gustatory nerve responses after lingual treatment of Gur in WT mice. We used agonists for mGluR1 (Quis: a group I mGluR agonist) and mGluR4 (L-AP4: a group III mGluR agonist), which cover EC_{50} of these agonists in brain or synapses (Fig. 4). The following concentrations of each agonist were tested: 0.1, 1, 10, 100, 300 μM, and 1, 3 mM. For both agonists tested, significant responses were detected at 1 and 3 mM in the CT and at 100 μM, 300 μM and 1, 3 mM in the GL (*t* test, Table 3). However, it is difficult to evaluate the effect of

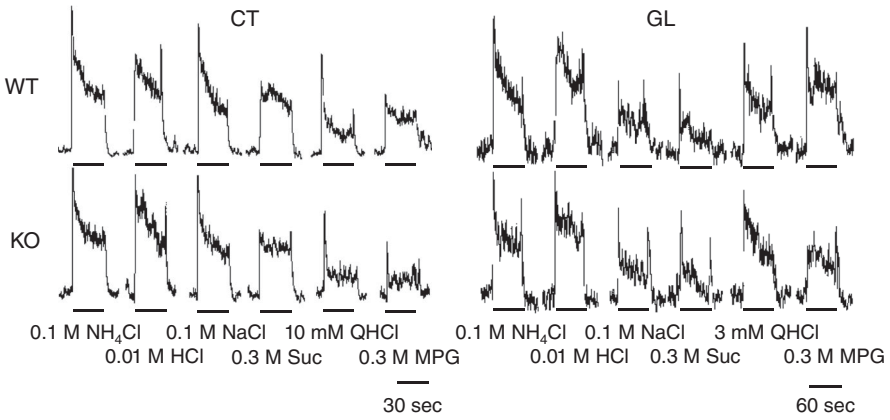

Figure 1. Sample recordings of integrated whole nerve responses from the CT and the GL nerve of a WT (upper trace) and mGluR4-KO mouse (lower trace)
Taste stimuli were 0.1 M NH_4Cl, 0.01 M HCl, 0.1 M NaCl, 0.3 M Suc, 10 mM QHCl (for CT), 3 mM QHCl (for GL) and 0.3 M MPG. Bars indicate application of taste stimuli. CT, chorda tympani; GL, glossopharyngeal; KO, knockout; MPG, monopotassium glutamate; QHCl, quinine HCl; Suc, sucrose; WT, wild-type.

antagonists particularly in the CT by using small responses to 1 mM agonist. Therefore, we focused on nerve responses to 3 mM of agonists that would activate taste-type mGluR. As shown in Fig. 5, both agonists (3 mM) for mGluR1 and mGluR4 with or without 0.5 mM IMP elicited responses of the CT and GL nerves in WT mice (*t* test comparing neural activities before and after stimulation onset; Table 4). As compared to WT mice, mGluR4-KO mice showed a significant reduction of responses of the CT and GL nerves to L-AP4 with and without IMP, but not to mGluR1 agonists with and without IMP (*t* test, Table 5, Fig. 5), suggesting involvement of mGluR4 in taste responses to L-AP4 in both the anterior and posterior tongue of mice. CT nerve responses to the agonists were potentiated by the addition of IMP (Fig. 5 and Table 6), indicating that part of CT nerve responses to both agonists used might be mediated by T1R1 + T1R3. To block the responses mediated by T1R1 + T1R3, a selective T1R3 blocker Gur was treated on the mouse tongue. After Gur treatment, CT nerve responses to agonists with and without IMP were significantly reduced (*t* test, $P < 0.05$, Table 7, Fig. 5). In the GL nerve, no such reduction of responses after Gur was observed (*t* test, $P > 0.1$; Table 7, Fig. 5). In mGluR4-KO mice, Quis and Quis + IMP elicited both CT and GL nerve responses after treatment of Gur (*t* test, $P < 0.05$; Table 4, Fig. 5), indicating that a part of CT and GL nerve responses to Quis with and without IMP may be mediated by other than T1R1 + T1R3 and mGluR4.

Agonist with antagonist experiment

Next, we recorded CT and GL nerve responses of mGluR4-KO mice to 300 mM MPG and MPG + IMP with concentration series of antagonists after treatment with Gur. When responses to a mixture of tastant and antagonist were compared with the sum of responses to each of the compounds, AIDA significantly suppressed CT responses to 300 mM MPG and MPG + IMP in a dose-dependent manner (repeated measures ANOVA: effect of antagonist, $P < 0.05$; effect of concentration, $P < 0.05$; Fig. 6, Table 8). In addition, AIDA significantly suppressed GL responses to 300 mM MPG (repeated measures ANOVA: effect of antagonist, $P < 0.05$; effect of concentration, $P < 0.05$; Fig. 6, Table 8). At the highest concentration of AIDA (3 mM), responses to 300 mM MPG were reduced to

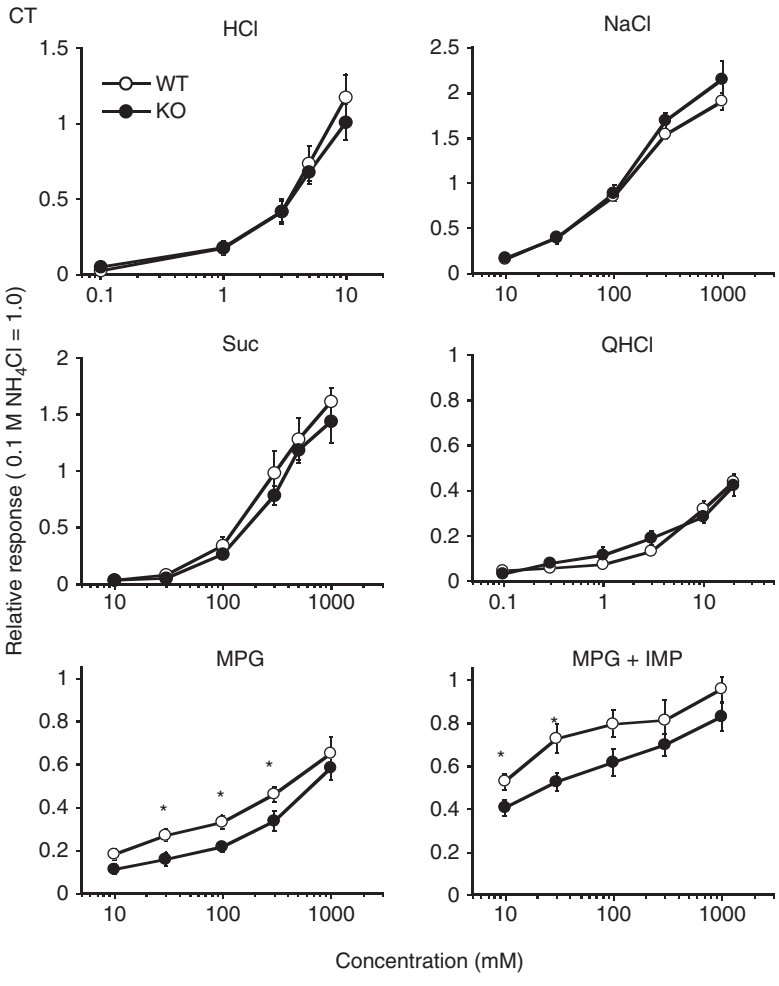

Figure 2. Relative responses to concentration series of HCl, NaCl, Suc, QHCl, MPG, MPG with 0.5 mM IMP (MPG + IMP) in the CT nerves of WT and mGluR4-KO mice
Response to 0.1 M NH_4Cl was used as a unity (1.0). Values indicated are means ± SEM. Responses to MPG with and without IMP in mGluR4-KO mice were significantly different from those in WT mice (repeated measures ANOVA, Table 1). Post hoc *Fisher* test; $^*P < 0.05$. CT, chorda tympani; IMP, inosine monophosphate; KO, knockout; MPG, monopotassium glutamate; QHCl, quinine HCl; Suc, sucrose; WT, wild-type.

50–80% of control responses in the CT and GL nerves. In contrast, CPPG did not affect responses to MPG and MPG + IMP in both the CT and GL nerves (Fig. 6, Table 8).

In addition, we used mGluR agonists (Quis and L-AP4) and various glutamate receptor antagonists (AIDA, CPPG, D-AP5 and NBQX) to test whether responses to each of the agonists are specifically inhibited by the corresponding antagonist in WT mice (Fig. 7, Table 9). A statistically significant difference was detected by one-way ANOVA in response to 3 mM Quis with and without antagonists in the CT and GL nerve (Fig. 7, Table 9). Responses to Quis with AIDA were significantly smaller than responses to Quis with and without other antagonists in the CT and GL nerve (*post-hoc* Bonferroni test, $P < 0.05$; Fig. 7). Concerning L-AP4, a statistically significant difference was detected by one-way ANOVA in response to 3 mM L-AP4 with and without antagonists in the CT and GL nerve (Fig. 7, Table 9). Responses to L-AP4 with CPPG were significantly smaller than responses to L-AP4 with other antagonists in the CT and GL nerve (*post-hoc* Bonferroni test, $P < 0.01$; Fig. 7). No statistically significant responses

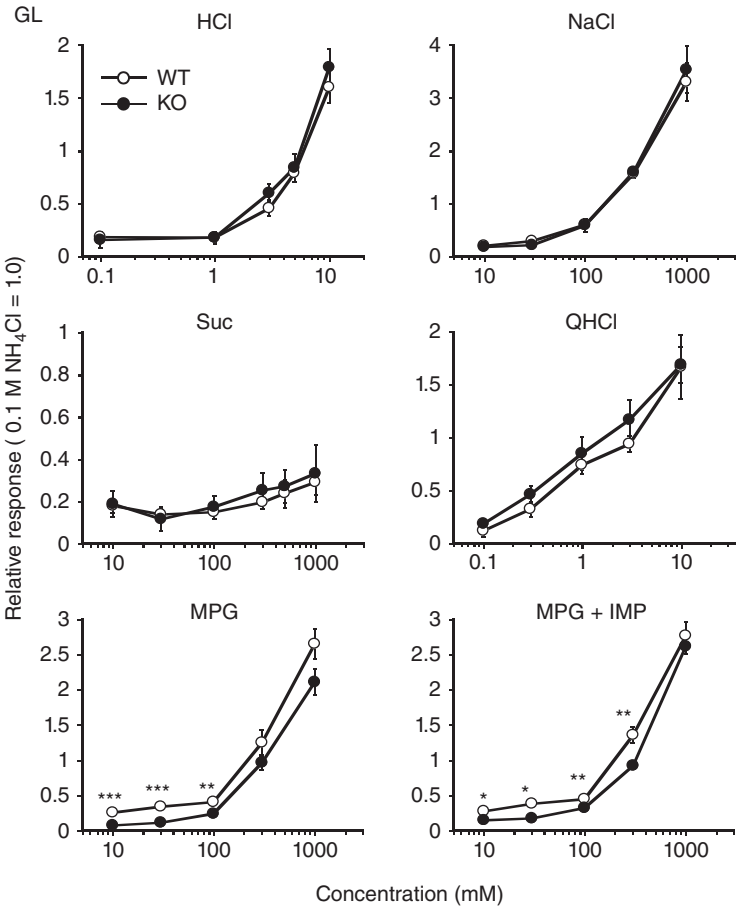

Figure 3. Relative responses to concentration series of HCl, NaCl, Suc, QHCl, MPG, MPG with 0.5 mM IMP (MPG + IMP) in the GL nerves of WT and mGluR4-KO mice
Response to 0.1 M NH_4Cl was used as a unity (1.0). Values indicated are means ± SEM. Responses to MPG with and without IMP in mGluR4-KO mice were significantly different from those in WT mice (repeated measures ANOVA, Table 1). Post hoc *Fisher* test; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. GL, glossopharyngeal; IMP, inosine monophosphate; KO, knockout; MPG, monopotassium glutamate; QHCl, quinine HCl; Suc, sucrose; WT, wild-type.

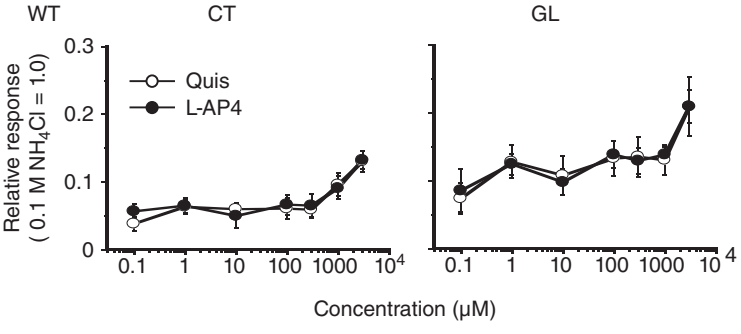

Figure 4. Relative responses to concentration series of Quis and L-AP4 after lingual treatment of Gur in the CT (left) and GL (right) nerves of WT mice
Response to 0.1 M NH_4Cl was used as a unity (1.0). Values indicated are means ± SEM. In the CT, significant responses were statistically detected in 1 and 3 mM Quis and 1 and 3 mM L-AP4 (*t* test, Table 3). In the GL, significant responses were statistically detected in 100 μM–3 mM Quis and 100 μM–3 mM L-AP4 (Table 3). L-AP4, L(+)-2-amino-4-phosphonobutyrate; CT, chorda tympani; GL, glossopharyngeal; Gur, gurmarin; Quis, quisquaric acid; WT, wild-type.

to antagonists were detected in the CT and GL of WT mice (Fig. 7, Table 10). These data indicate that mGluR agonists and antagonists used may be relatively selective to their targets at least as related to group I *vs.* group III mGluR even though we used high concentration of agonists and antagonists. The CT nerve responses to other taste stimuli (NaCl, HCl, QHCl and sucrose) in mGluR4-KO mice were not affected by all antagonists (one-way ANOVA: $F_{4,32} = 0.269$, $P = 0.896$ for NaCl; $F_{4,32} = 0.348$, $P = 0.844$ for HCl; $F_{4,39} = 0.642$, $P = 0.643$ for QHCl; $F_{4,28} = 0.227$, $P = 0.921$ for sucrose; Fig. 8). Taken together, these results suggest that mGluR4, T1R1 + T1R3 and group I mGluR (mGluR1 or mGluR5) may be involved in umami taste responses on the anterior tongue and mGluR4 and the group I mGluR receptors may be involved in the umami responses on the posterior tongue.

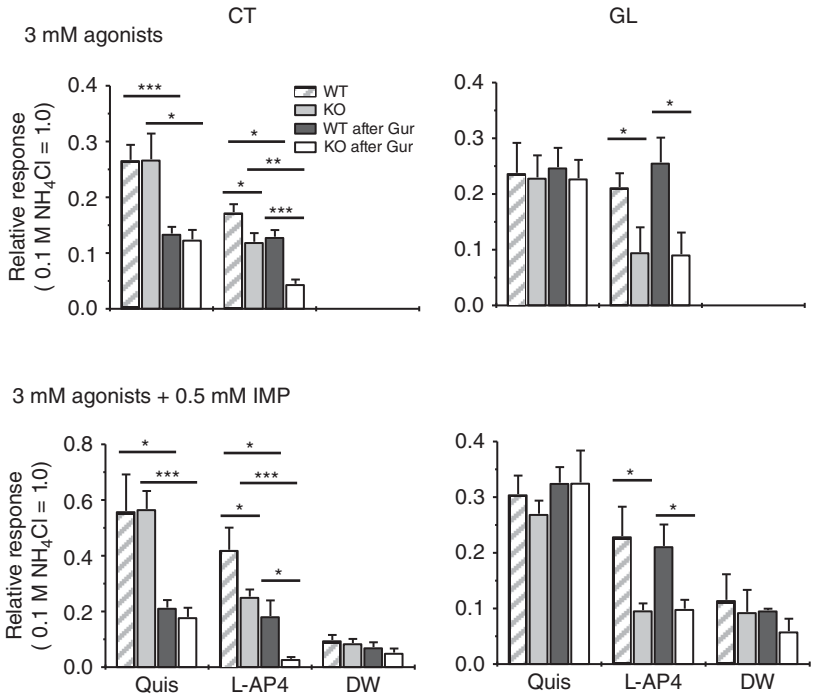

Figure 5. Relative responses to 3 mM agonists for group I mGluR (Quis) and group III mGluR (L-AP4) with (lower) or without 0.5 mM IMP (upper) and 0.5 mM IMP only (lower) before and after lingual treatment of Gur in the CT and GL nerves of WT and mGluR4-KO mice
Response to 0.1 M NH_4Cl was used as a unity (1.0). Values indicated are means ± SEM. Responses to agonists with and without IMP were significantly inhibited by Gur in the CT (Table 7). Responses to L-AP4 with and without IMP in mGluR4-KO mice were significantly different from those in WT mice (Table 5). Student's *t* test; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. L-AP4, L(+)-2-amino-4-phosphonobutyrate; CT, chorda tympani; DW, distilled water; GL, glossopharyngeal; Gur, gurmarin; IMP, inosine monophosphate; KO, knockout; Quis, quisquaric acid; WT, wild-type.

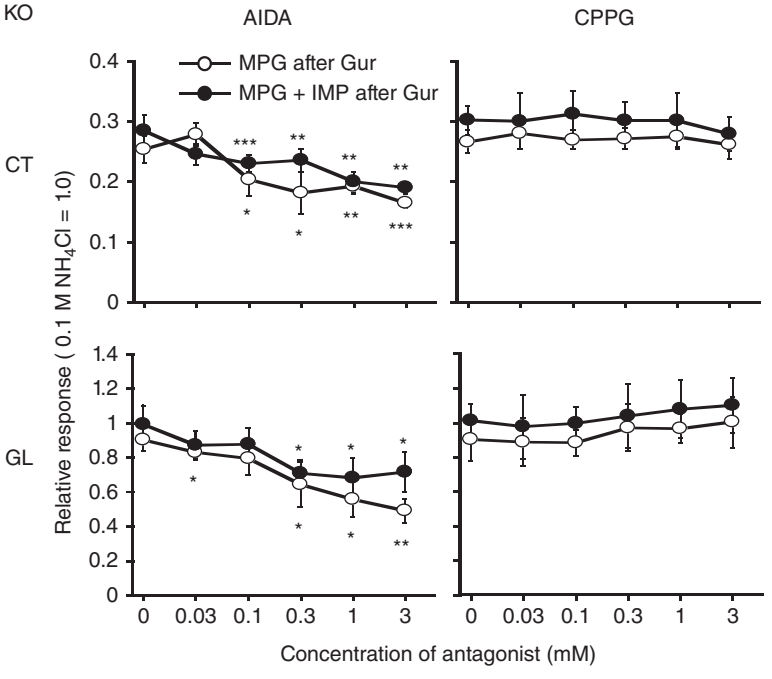

Figure 6. Dose-dependent effect of AIDA and CPPG on responses to 0.3 M MPG (white symbols) or 0.3 M MPG + 0.5 mM IMP (black symbols) in the CT (upper) and the GL nerves (lower) of mGluR4-KO mice
Gur was applied to the tongue before recording responses. Response to 0.1 M NH_4Cl was used as a unity (1.0). Values indicated are means ± SEM. The sum of responses to 0.3 M MPG and AIDA (in the CT and GL) is significantly different from response to the mixture (repeated measures ANOVA, Table 8). Post hoc *Fisher* test; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. AIDA, (*RS*)-1-aminoindan-1,5-dicarboxylic acid; CPPG, (*RS*)-alpha-cyclopropyl-4-phosphonophenylglycine; CT, chorda tympani; GL, glossopharyngeal; Gur, gurmarin; IMP, inosine monophosphate; KO, knockout; MPG, monopotassium glutamate.

Table 4. Results of Student's *t* test for the occurrence of responses to agonists for glutamate receptors in the CT and GL of WT and mGluR4-KO mice (Fig. 5)

Agonist	WT	KO
CT nerve		
Quis	$t_{18} = 3.645$, $P = 0.005$	$t_{18} = 3.732$, $P < 0.001$
Quis after Gur	$t_{18} = 3.781$, $P = 0.001$	$t_{18} = 4.650$, $P < 0.001$
Quis + IMP	$t_{18} = 4.192$, $P < 0.001$	$t_{18} = 8.912$, $P < 0.001$
Quis + IMP after Gur	$t_{18} = 4.116$, $P < 0.001$	$t_{18} = 4.214$, $P < 0.001$
L-AP4	$t_{18} = 2.606$, $P = 0.018$	$t_{18} = 2.589$, $P = 0.019$
L-AP4 after Gur	$t_{18} = 4.184$, $P < 0.001$	$t_{18} = 1.227$, $P = 0.235$
L-AP4 + IMP	$t_{18} = 4.005$, $P < 0.001$	$t_{18} = 6.870$, $P < 0.001$
L-AP4 + IMP after Gur	$t_{18} = 6.214$, $P < 0.001$	$t_{18} = 1.641$, $P = 0.118$
0.5 mM IMP	$t_{18} = 1.731$, $P = 0.101$	$t_{18} = 0.291$, $P = 0.774$
0.5 mM IMP after Gur	$t_{18} = 0.560$, $P = 0.582$	$t_{18} = 0.454$, $P = 0.655$
GL nerve		
Quis	$t_{18} = 4.969$, $P < 0.001$	$t_{18} = 4.591$, $P < 0.001$
Quis after Gur	$t_{18} = 2.218$, $P = 0.040$	$t_{18} = 2.214$, $P = 0.040$
Quis + IMP	$t_{18} = 3.749$, $P = 0.002$	$t_{18} = 3.241$, $P = 0.005$
Quis + IMP after Gur	$t_{18} = 4.079$, $P < 0.001$	$t_{18} = 2.752$, $P = 0.013$
L-AP4	$t_{18} = 3.279$, $P = 0.006$	$t_{18} = 1.279$, $P = 0.224$
L-AP4 after Gur	$t_{18} = 3.887$, $P = 0.001$	$t_{18} = 0.653$, $P = 0.522$
L-AP4 + IMP	$t_{18} = 2.226$, $P = 0.039$	$t_{18} = 0.005$, $P = 0.996$
L-AP4 + IMP after Gur	$t_{18} = 3.615$, $P = 0.002$	$t_{18} = 0.241$, $P = 0.812$
0.5 mM IMP	$t_{18} = 1.246$, $P = 0.229$	$t_{18} = 0.971$, $P = 0.345$
0.5 mM IMP after Gur	$t_{18} = 1.147$, $P = 0.267$	$t_{18} = 0.297$, $P = 0.770$

Abbreviations: L-AP4, L(+)-2-amino-4-phosphonobutyrate; CT, chorda tympani; GL, glossopharyngeal; Gur, gurmarin; IMP, inosine monophosphate; KO, knockout; Quis, quisquaric acid; WT, wild-type. Significant responses were detected based on the comparison of neural activities between before and after stimulation onset with each agonist. *t* test.

Table 5. Results of Student's *t* test for the effect of mGluR4 gene on responses to agonists for glutamate receptors in the CT and the GL (WT *vs.* mGluR4-KO mice; Fig. 5)

Agonist	CT	GL
Quis	$t_{15} = 0.017$, $P = 0.986$	$t_{13} = 0.112$, $P = 0.912$
Quis after Gur	$t_{10} = 0.504$, $P = 0.625$	$t_{10} = 0.413$, $P = 0.688$
Quis + IMP	$t_{10} = 0.01$, $P = 0.960$	$t_{12} = 0.910$, $P = 0.115$
Quis + IMP after Gur	$t_{9} = 0.761$, $P = 0.466$	$t_{8} = 0.586$, $P = 0.574$
L-AP4	$t_{13} = 2.307$, $P = 0.038$	$t_{12} = 2.511$, $P = 0.027$
L-AP4 after Gur	$t_{8} = 5.903$, $P < 0.001$	$t_{10} = 2.790$, $P = 0.019$
L-AP4 + IMP	$t_{10} = 2.253$, $P = 0.048$	$t_{10} = 2.466$, $P = 0.045$
L-AP4 + IMP after Gur	$t_{7} = 3.103$, $P = 0.017$	$t_{8} = 2.710$, $P = 0.027$

Abbreviations: L-AP4, L(+)-2-amino-4-phosphonobutyrate; CT, chorda tympani; GL, glossopharyngeal; Gur, gurmarin; IMP, inosine monophosphate; KO, knockout; Quis, quisquaric acid; WT, wild-type.

Discussion

To date, umami responses have been analysed using the KO model mice, which have a deletion in *Tas1r* family genes. Mice lacking the entire *Tas1r1* coding region and the T1R3 null mice showed a severe reduction in the synergistic response to glutamate and IMP in the CT nerve (Damak *et al.* 2003; Yasumatsu *et al.* 2012; Kusuhara *et al.* 2013). Contrary to the CT, GL nerve responses of the T1R1-KO and T1R3-KO mice to glutamate and glutamate with IMP did not differ significantly from those of the WT mice (Damak *et al.* 2003; Kusuhara *et al.* 2013). On the other hand, Zhao *et al.* (2003) showed that CT nerve responses to glutamate were totally abolished in both T1R1-KO and T1R3-KO models. The differences between the two different T1R3-KO models and how they were analysed may contribute to some of these differences in nerve responses to umami stimuli. The T1R3-KO model of Damak *et al.* lacks the entire T1R3 coding region, the gene's promoter and expresses no T1R3 protein. This mouse was

generated in C57BL/6J embryonic stem cells, maintained in that background and was compared to littermates of the same C57BL/6J background. The T1R3-KO model of Zhao *et al.* deleted the amino-terminal extracellular domain but not the seven transmembrane helices of T1R3. This mouse was generated in 129 embryonic stem cells, backcrossed for two generations with C57BL/6 mice and compared to 129X1/SvJ and C57BL/6 mice. These differences in methods as well as strain background might affect the outcome of the result between two groups. In the present study, we demonstrated that another KO model mouse, mGluR4-KO, showed a selective and significant reduction of responses to umami compounds and L-AP4 (mGluR4 agonist) without affecting responses to sour, salty, sweet, bitter compounds and mGluR1 agonist in both the CT and GL nerve (Figs 1–3 and 5, Tables 1, 4 and 5). Our data obtained from mGluR4-KO mice are comparable with previous reports using the mGluR4 antagonist, CPPG, which selectively inhibited glutamate responses in single taste cells of rats (Lin & Kinnamon, 1999) and B6 mice (Kusuhara *et al.* 2013), in the CT and GL nerve of $T1R1^{-/-}$ and $T1R1^{+/-}$ mice (Kusuhara *et al.* 2013), in a subset of CT fibres of B6 mice (Yasumatsu *et al.* 2012), and in behavioural tests of rats (Eschle *et al.* 2009) and mice

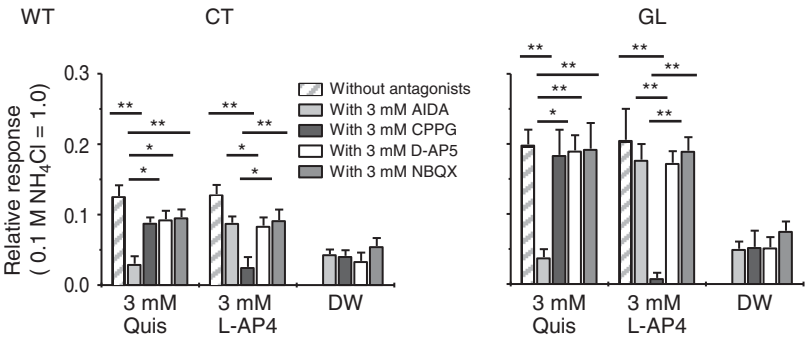

Figure 7. Relative responses to Quis and L-AP4 with antagonists for glutamate receptors and antagonists alone after lingual treatment of Gur in the CT (left) and the GL (right) nerves of WT mice
Antagonists tested were AIDA, CPPG, D-AP5 (antagonist for NMDA receptor) and NBQX (antagonist for AMPA and kainic acid receptors). Concentrations tested were 3 mM for all agonists and antagonists. Response to 0.1 M NH_4Cl was used as a unity (1.0). Values indicated are means ± SEM. Responses to Quis with AIDA and L-AP4 with CPPG were significantly different from those to other mixtures of agonists with antagonists in the CT and GL (one-way ANOVA, *post hoc Bonferroni* test, Table 9, $*P < 0.05$; $**P < 0.01$). Responses to antagonists were not significantly detected (Table 10). AIDA, (*RS*)-1-aminoindan-1,5-dicarboxylic acid; D-AP5, D-(–)-2-amino-5-phosphonopentanoic acid; CPPG, (*RS*)-alpha-cyclopropyl-4-phosphonophenylglycine; CT, chorda tympani; DW, distilled water; GL, glossopharyngeal; Gur, gurmarin; IMP, inosine monophosphate; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulphonamide; Quis, quisquaric acid; WT, wild-type.

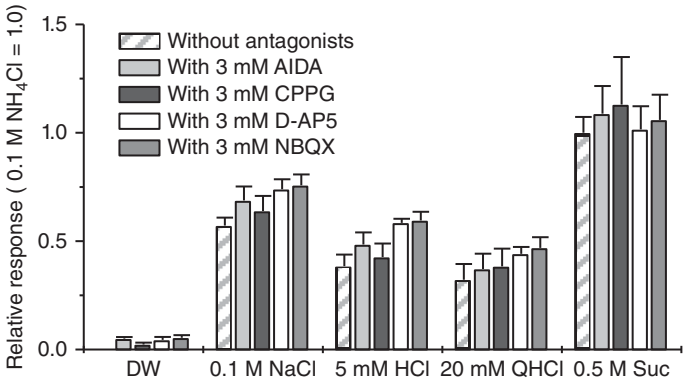

Figure 8. The effect of antagonists for glutamate receptors on responses to basic taste stimuli in the chorda tympani of mGluR4-KO mice
Basic taste stimuli are 0.1 M NaCl, 5 mM HCl, 20 mM QHCl and 0.5 M Suc. Columns indicate responses to DW or basic taste stimuli with and without antagonists (3 mM AIDA, 3 mM CPPG, 3 mM D-AP5 and 3 mM NBQX). Response to 0.1 M NH_4Cl was used as a unity (1.0). Values indicated are means ± SEM. There was no significant difference in factor of antagonists (one-way ANOVA, $P > 0.05$). AIDA, (*RS*)-1-aminoindan-1,5-dicarboxylic acid; D-AP5, D-(–)-2-amino-5-phosphonopentanoic acid; CPPG, (*RS*)-alpha-cyclopropyl-4-phosphonophenylglycine; DW, distilled water; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulphonamide; QHCl, qinine HCl; Suc, sucrose.

Table 6. Results of paired *t* test for the effect of addition of IMP on responses to agonists for glutamate receptors in the CT and GL of the WT and mGluR4-KO mice (Fig. 5)

Agonist	WT	KO
CT nerve		
Quis	$t_5 = 3.034$, $P = 0.029$	$t_5 = 3.959$, $P = 0.011$
Quis after Gur	$t_4 = 4.720$, $P = 0.009$	$t_5 = 2.781$, $P = 0.039$
L-AP4	$t_6 = 3.210$, $P = 0.018$	$t_4 = 5.668$, $P = 0.005$
L-AP4 after Gur	$t_4 = 1.380$, $P = 0.240$	$t_4 = 1.025$, $P = 0.363$
GL nerve		
Quis	$t_6 = 4.760$, $P = 0.003$	$t_5 = 3.839$, $P = 0.012$
Quis after Gur	$t_4 = 4.995$, $P = 0.008$	$t_5 = 2.620$, $P = 0.047$
L-AP4	$t_5 = 0.298$, $P = 0.778$	$t4 = 0.022$, $P = 0.983$
L-AP4 after Gur	$t_4 = 0.581$, $P = 0.592$	$t_4 = 0.194$, $P = 0.856$

Abbreviations: L-AP4, L(+)-2-amino-4-phosphonobutyrate; CT, chorda tympani; GL, glossopharyngeal; Gur, gurmarin; IMP, inosine monophosphate; KO, knockout; Quis, quisquaric acid; WT, wild-type. Differences between agonists with and without IMP were assessed by paired *t* test in CT (upper) and GL (lower).

Table 7. Results of Student's *t* test for the effect of Gur on responses to agonists for glutamate receptors in the CT and GL of WT and mGluR4-KO mice (Fig. 5)

Agonist	WT	KO
CT nerve		
Quis	$t_{15} = 3.488$, $P = 0.003$	$t_{10} = 2.917$, $P = 0.015$
Quis + IMP	$t_9 = 2.592$, $P = 0.046$	$t_{10} = 5.379$, $P < 0.001$
L-AP4	$t_{13} = 2.370$, $P = 0.035$	$t_8 = 4.347$, $P = 0.003$
L-AP4 + IMP	$t_9 = 2.268$, $P = 0.049$	$t_9 = 8.426$, $P < 0.001$
GL nerve		
Quis	$t_{13} = 0.139$, $P = 0.892$	$t_{10} = 0.029$, $P = 0.977$
Quis + IMP	$t_{11} = 0.804$, $P = 0.439$	$t_9 = 0.122$, $P = 0.906$
L-AP4	$t_{13} = 0.940$, $P = 0.364$	$t_9 = 0.069$, $P = 0.946$
L-AP4 + IMP	$t_{10} = 0.251$, $P = 0.807$	$t_8 = 0.507$, $P = 0.626$

Abbreviations: L-AP4, L(+)-2-amino-4-phosphonobutyrate; CT, chorda tympani; GL, glossopharyngeal; Gur, gurmarin; IMP, inosine monophosphate; KO, knockout; Quis, quisquaric acid; WT, wild-type.

(Nakashima *et al.* 2001, 2012; Kusuhara *et al.* 2013). Thus, the present study provided functional evidence for the involvement of mGluR4 in normal umami taste responses in mice.

In our previous study (Yasumatsu *et al.* 2012), glutamate-responding CT fibres could be classified into two types: sucrose-best (S-type) and MPG-best (M-type), and each group can be further subdivided into two subtypes, with (S1- and M1-type) or without umami synergism (S2- and M2-type). Among them, S1-type is characterized by robust responses to sweet compounds and large synergism of glutamate responses by IMP. In T1R3-KO mice, only S1-type fibres were absent. Therefore, it was suggested that S1-type fibres might receive taste information from taste cells that express both sweet receptor T1R2 + T1R3, and umami receptor T1R1 + T1R3. The responses to glutamate in M1-type and in M2-type fibres were suppressed by AIDA and CPPG, respectively. Thus, M1- and M2-type fibres may receive taste information from taste cells that express mGluR1 and mGluR4, respectively. In addition, responses to glutamate and L-AP4 in M2-type fibres were not enhanced by IMP (Yasumatsu *et al.* 2012). Glutamate-sensitive taste cells in the taste buds of FP were also classified into S1-, S2-, M1- and M2-types, suggesting that each cell type transmits glutamate signals to corresponding type of gustatory nerve fibres (Niki *et al.* 2011). The present study demonstrated that L-AP4 did not elicit significant responses in both the CT and GL nerves after Gur (Fig. 5, Table 4) and MPG responses were not affected by CPPG (Fig. 6, Table 8) in mGluR4-KO mice. Such an eliminated component of umami responses would be mediated by mGluR4 and M2-type cells and fibres would be responsible for these responses. We also found that CT and GL nerve responses to Quis and suppression of MPG response by AIDA were not affected by deletion of the mGluR4 gene (Figs 5 and 6, Tables 4–6 and 8), indicating that mGluR4 did not contribute to these responses, which would be mediated by mGluR1 and M1-type cells and fibres. In addition, we demonstrated that large synergistic responses to glutamate and agonists for mGluR with IMP, which would be mediated by T1R1 + T1R3 and S1-type cells and fibres, were still observed in mGluR4-KO mice (Figs 2 and 5, Tables 2 and 6). All these findings further support the above-mentioned hypothesis that mGluR4 contributes to umami taste responses via M2-type taste cells and nerve fibres but not via M1- or S-types and suggest the existence of multiple receptors and transduction systems for umami taste. The present study showed residual responses to MPG with and without IMP after suppression of the responses via mGluR1 and T1R3 by AIDA and Gur respectively in mGluR4-KO mice (Fig. 6). These residual responses might include responses to the K^+ component of MPG and presumably responses to glutamate (with IMP) via T1R1 or an unidentified protein as suggested by Smith and Spector (2014). Insufficient blockade of T1R3-dependent receptor by Gur also may contribute to these residual responses, but this is unlikely because the synergistic response, which would be mediated by T1R1 + T1R3, was not observed in these residual responses (Fig. 6).

Regarding responses to umami substances, differential responsiveness between the CT and the GL nerve has been suggested in mice (Ninomiya & Funakoshi, 1989; Ninomiya *et al.* 1991) and rhesus monkeys (Hellekant *et al.* 1997). They showed CT fibres that responded to both glutamate (MSG with and without disodium 5′-guanylate) and sucrose in mice (Ninomiya & Funakoshi, 1989) and rhesus monkeys (Hellekant *et al.* 1997). These fibres may

Table 8. Repeated measures ANOVA results for the effect of concentration series of antagonists on responses to 300 mM MPG with and without IMP in the CT and GL of mGluR4-KO mice (Fig. 6)

Nerve	Antagonists CT (upper), GL (lower)	Concentration CT (upper), GL (lower)	Antagonists × Concentration CT (upper), GL (lower)
AIDA + MPG	$F_{1,32} = 11.005$, $P = 0.011$	$F_{4,32} = 5.012$, $P = 0.003$	$F_{4,32} = 3.422$, $P = 0.019$
	$F_{1,40} = 10.392$, $P = 0.009$	$F_{4,40} = 3.449$, $P = 0.016$	$F_{4,40} = 5.821$, $P < 0.001$
AIDA + MPG + IMP	$F_{1,40} = 7.697$, $P = 0.020$	$F_{4,40} = 5.833$, $P < 0.001$	$F_{4,40} = 5.204$, $P = 0.002$
	$F_{1,40} = 10.600$, $P = 0.009$	$F_{4,40} = 1.459$, $P = 0.233$	$F_{4,40} = 3.055$, $P = 0.028$
CPPG + MPG	$F_{1,40} = 4.111$, $P = 0.070$	$F_{4,40} = 0.229$, $P = 0.921$	$F_{4,40} = 0.557$, $P = 0.695$
	$F_{1,40} = 0.002$, $P = 0.963$	$F_{4,40} = 1.168$, $P = 0.339$	$F_{4,40} = 1.009$, $P = 0.414$
CPPG + MPG + IMP	$F_{1,40} = 1.444$, $P = 0.257$	$F_{4,40} = 0.802$, $P = 0.531$	$F_{4,40} = 1.538$, $P = 0.209$
	$F_{1,40} = 0.007$, $P = 0.937$	$F_{4,40} = 0.613$, $P = 0.656$	$F_{4,40} = 0.640$, $P = 0.637$

Abbreviations: AIDA, (*RS*)-1-aminoindan-1,5-dicarboxylic acid; CPPG, (*RS*)-alpha-cyclopropyl-4-phosphonophenylglycine; CT, chorda tympani; GL, glossopharyngeal; IMP, inosine monophosphate; KO, knockout; MPG, monopotassium glutamate. Differences between mixture of antagonists with MPG or MPG + IMP and the sum of each component were assessed by repeated measures ANOVA.

correspond to the S1-type (Yasumatsu *et al.* 2012) and the Gur-sensitive component in response to agonists with and without IMP in the current study, whereas these fibres were not found in the GL (Ninomiya & Funakoshi, 1989; Hellekant *et al.* 1997). They showed that GL fibres responding to glutamate did not respond to sweet substances. In the current study, responses to agonists were greater in the GL than in the CT nerve after lingual treatment of Gur (Figs 5 and 7). Additionally, responses to agonists were not suppressed by Gur in the GL nerve as previously reported in sweet taste (Ninomiya *et al.* 1997). These results suggest that the expression levels of mGluR1 and mGluR4 are different between the anterior and posterior of the tongue and that T1R1 + T1R3 functions mainly on the anterior tongue.

Although expression of T1R3 was prominent in FP, foliate and CV taste buds, the expression of T1R1 was not clear in CV and/or foliate taste buds (Kitagawa *et al.* 2001; Montmayeur *et al.* 2001; Nelson *et al.* 2001). Kim *et al.* (2003) detected T1R1 in CV taste buds. However, the expression level of T1R1 in CV taste buds was lower than that in FP taste buds. Therefore, the difference in expression levels between T1R1 and T1R3 may be one of the reasons for the lack of function of T1R1 + T1R3 heterodimers on the posterior of the tongue. However, we observed significant enhancement of responses to Quis by IMP in the GL nerve (Fig. 5, Table 6). If T1R1 + T1R3 heterodimers did not contribute to this enhancement, other receptors including mGluR1 might also contribute to the occurrence of synergistic responses. To elucidate this possibility, further studies are required. Concerning expression of mGluR, Toyono *et al.* (2003, 2002) found that mGluR1 and mGluR4 proteins are localized in the apical side of taste cells of FP, foliate and CV taste buds of rats. There is no report that indicates a difference among taste papillae in expression levels of mGluR1 and mGluR4 and that indicates the difference between brain-type and taste-type in expression levels of mGluR1 and mGluR4. However, the present study demonstrated that taste-mGluR1 and taste-mGluR4 might play more important roles than brain-type mGluR in the detection of glutamate in both the anterior and posterior of the tongue.

Table 9. One-way ANOVA results for the effects of antagonists on responses to agonists for glutamate receptors after gurmarin treatment in the CT and GL of wild-type mice (Fig. 7)

Nerve stimulus	CT	GL
Quisquaric acid	$F_{4,24} = 8.313$, $P < 0.001$	$F_{4,21} = 6.446$, $P = 0.002$
L-AP4	$F_{4,24} = 8.249$, $P < 0.001$	$F_{4,21} = 10.104$, $P < 0.001$

Abbreviations: L-AP4, L(+)-2-amino-4-phosphonobutyrate; CT, chorda tympani; GL, glossopharyngeal.

Table 10. Results of Student's *t* test for the occurrence of responses to 3 mM antagonists for glutamate receptors in the CT and GL of wild-type mice (Fig. 7)

	CT nerve	GL nerve
AIDA	$t_{18} = 1.364$, $P = 0.190$	$t_{18} = 0.102$, $P = 0.920$
CPPG	$t_{18} = 0.176$, $P = 0.862$	$t_{18} = 0.122$, $P = 0.904$
D-AP5	$t_{18} = 0.680$, $P = 0.505$	$t_{18} = 1.168$, $P = 0.258$
NBQX	$t_{18} = 1.316$, $P = 0.205$	$t_{18} = 1.687$, $P = 0.109$

Abbreviations: AIDA, (*RS*)-1-aminoindan-1,5-dicarboxylic acid; D-AP5, D-(–)-2-amino-5-phosphonopentanoic acid; CPPG, (*RS*)-alpha-cyclopropyl-4-phosphonophenylglycine; CT, chorda tympani; GL, glossopharyngeal; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulphonamide. Significant responses were detected based on the comparison of neural activities between before and after stimulation onset with each agonist. *t* test.

When considering relevance to behavioural studies, the present study is consistent with many reports. Although results differed among studies whether T1R3-KO mice are able to detect sucrose at concentrations comparable to those detected by WT mice or not (Delay *et al.* 2006; Treesukosol & Spector, 2012), Delay *et al.* showed that T1R3-KO mice were able to discriminate between the taste qualities of MSG and sucrose, even when the cue function of the sodium component of MSG was reduced. Conditioned taste aversion tests demonstrated that both $T1R1^{-/-}$ and $T1R1^{+/-}$ mice were equally capable of discriminating glutamate from other basic taste stimuli (Kusuhara *et al.* 2013). These results suggest that the T1R1-KO and the T1R3-KO mice used T1R1- and T1R3-independent mechanisms to detect glutamate. In respect of preference, T1R3-KO or T1R1-KO mice showed no preference for 3–100 mM MSG + IMP by short lick tests (Zhao *et al.* 2003) and, T1R3-KO mice showed no preference for 1–30 mM MSG by 48 h two-bottle preference tests (Damak *et al.* 2003). In mGluR4-KO mice, the preference for glutamate solutions was more pronounced than in the WT mice (Chaudhari & Roper, 1998). This result may indicate that taste information, which is initiated by mGluR4, is not a signal for preference. All these reports together, taste information detected by T1R1 + T1R3 may play a role for preference and those detected by mGluR1 and mGluR4 may play roles in the discrimination of taste qualities and/or in other processes associated with feeding. However, to test this hypothesis, further studies using mice models lacking both mGluR1 and mGluR4 would be required.

In conclusion, the present study provided functional evidences for the contribution of mGluR4 in umami taste information in taste cells of both the anterior and posterior of the tongue. Moreover, we demonstrated gustatory nerve responses to agonists for mGluR1 and -4 and inhibition of gustatory nerve responses to glutamate and mGluR agonists by Gur and mGluR antagonists, indicating the involvement of multiple taste receptors (T1R1 + T1R3, mGluR1 and mGluR4) in umami taste information. The differential expression levels of these multiple umami taste receptors may underlie the differential umami responsiveness and roles between the CT and GL nerve.

References

Behrens M, Meyerhof W, Hellfritsch C & Hofmann T (2011). Sweet and umami taste: natural products, their chemosensory targets, and beyond. *Angew Chem Int Ed Eng* **150**, 2220–2242

Chaudhari N, Landin AM & Roper SD (2000). A metabotropic glutamate receptor variant functions as a taste receptor. *Nat Neurosci* **3**, 113–119.

Chaudhari N, Pereira E & Roper SD (2009). Taste receptors for umami: the case for multiple receptors. *Am J Clin Nutr* **90**, 738S–742S.

Chaudhari N & Roper SD (1998). Molecular and physiological evidences for glutamate (umami) taste transduction via a G-protein coupled receptor. *Ann N Y Acad Sci* **855**, 398–406.

Chaudhari N, Yang H, Lamp C, Delay E, Cartford C, Than T & Roper SD (1996). The taste of monosodium glutamate: membrane receptors in taste buds. *J Neurosci* **16**, 3817–3826.

Daly K, Al-Rammahi M, Moran A, Marcello M, Ninomiya Y & Shirazi-Beechey SP (2013). Sensing of amino acids by the gut-expressed taste receptor T1R1-T1R3 stimulates CCK secretion. *Am J Physiol Gastrointest Liver Physiol* **304**, G271–G282.

Damak S, Rong M, Yasumatsu K, Kokrashvili Z, Varadarajan V, Zou S, Jiang P, Ninomiya Y & Margolskee RF (2003). Detection of sweet and umami taste in the absence of taste receptor T1R3. *Science* **301**, 850–853.

Delay ER, Hernandez NP, Bromley K & Margolskee RF (2006). Sucrose and monosodium glutamate taste thresholds and discrimination ability of T1R3 knockout mice. *Chem Senses* **31**, 351–357.

Eschle BK, Eddy MC & Delay ER (2009). Antagonism of metabotropic glutamate receptor 4 receptors by (RS)-alpha-cyclopropyl-4-phosphonophenylglycine alters the taste of amino acids in rats. *Neuroscience* **155**, 522–529.

Hellekant G, Danilova V & Ninomiya Y (1997). Primate sense of taste: behavioral and single chorda tympani and glossopharyngeal nerve fiber recordings in the rhesus monkey, Macaca mulatta. *J Neurophysiol* **77**, 978–993.

Kim MR, Kusakabe Y, Miura H, Shindo Y, Ninomiya Y & Hino A (2003). Regional expression patterns of taste receptors and gustducin in the mouse tongue. *Biochem Biophys Res Commun* **312**, 500–506.

Kitagawa M, Kusakabe Y, Miura H, Ninomiya Y & Hino A (2001). A molecular genetic identification of a candidate receptor gene for sweet taste. *Biochem Biophys Res Commun* **283**, 236–242.

Kurihara K & Kashiwayanagi M (2000). Physiological studies on umami taste. *J Nutrition* **130**, 931S-934S.

Kusuhara Y, Yoshida R, Ohkuri T, Yasumatsu K, Voigt A, Hübner S, Maeda K, Boehm U, Meyerhof W, & Ninomiya Y (2013). Taste responses in mice lacking taste receptor subunit T1R1. *J Physiol* **591**, 1967–1985.

Li X, Staszewski L, Xu H, Durick K, Zoller M & Adler E (2002). Human receptors for sweet and umami taste. *Proc Natl Acad Sci U S A* **99**, 4692–4696.

Lin W & Kinnamon SC (1999). Physiological evidence for ionotropic and metabotropic glutamate receptors in rat taste cells. *J Neurophysiol* **82**, 2061–2069.

Maruyama Y, Pereira E, Margolskee RF, Chaudhari N & Roper SD (2006). Umami responses in mouse taste cells indicate more than one receptor. *J Neurosci* **26**, 2227–2234.

Montmayeur JP, Liberles SD, Matsunami H, & Buck LB (2001). A candidate taste receptor gene near a sweet taste locus. *Nat Neurosci* **4**, 492–498.

Nakashima K, Eddy MC, Katsukawa H, Delay ER & Ninomiya Y (2012). Behavioral responses to glutamate receptor agonists and antagonists implicate the involvement of brain-expressed mGluR4 and mGluR1 in taste transduction for umami in mice. *Physiol Behav* **105**, 709–719.

Nakashima K, Katsukawa H, Sasamoto K & Ninomiya Y (2001). Behavioral taste similarities and differences among monosodium L-glutamate and glutamate receptor agonists in C57BL mice. *J Nutr Sci Vitaminol* **47**, 161–166.

Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ & Zuker CS (2002). An amino-acid taste receptor. *Nature* **416**, 199–202.

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ & Zuker CS (2001). Mammalian sweet taste receptors. *Cell* **106**, 381–390.

Niki M, Takai S, Kusuhara Y, Ninomiya Y & Yoshida R (2011). Responses to apical and basolateral application of glutamate in mouse fungiform taste cells with action potentials. *Cell Mol Neurobiol* **31**, 1033–1040.

Ninomiya Y (1998). Reinnervation of cross-regenerated gustatory nerve fibers into amiloride-sensitive and amiloride-insensitive taste receptor cells. *Proc Natl Acad Sci U S A* **95**, 5347–5350.

Ninomiya Y & Funakoshi M (1981). Responses of rat chorda tympani fibers to electrical stimulation of the tongue. *Jpn J Physiol* **31**, 559–570.

Ninomiya Y & Funakoshi M (1989). Peripheral neural basis for behavioural discrimination between glutamate and the four basic taste substances in mice. *Comp Biochem Physiol A* **92**, 371–376.

Ninomiya Y & Imoto T (1995). Gurmarin inhibition of sweet taste responses in mice. *Am J Physiol Regul Integr Comp Physiol* **268**, R1019–R1025.

Ninomiya Y, Tanimukai T, Yoshida S, & Funakoshi M (1991). Gustatory neural responses in preweanling mice. *Physiol Behav* **49**, 913–918.

Ninomiya Y, Inoue M, Imoto T & Nakashima K (1997). Lack of gurmarin sensitivity of sweet taste receptors innervated by the glossopharyngeal nerve in C57BL mice. *Am J Physiol Regul Integr Comp Physiol* **272**, R1002–R1006.

Pekhletski R, Gerali R, Overstreet LS, Huang XP, Agopyan N, Slater NT, Abramow-Newerly W, Roder JC & Hampson DR (1996). Impaired cerebellar synaptic plasticity and motor performance in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. *J Neurosci* **16**, 6364–6373.

San Gabriel A, Maekawa T, Uneyama H & Torii K (2009). Metabotropic glutamate receptor type 1 in taste tissue. *Am J Clin Nutr* **90**, 743S–746S.

San Gabriel A, Uneyama H, Yoshie S & Torii K (2005). Cloning and characterization of a novel mGluR1 variant from vallate papillae that functions as a receptor for L-glutamate stimuli. *Chem Senses* **30**, i25–i26.

Smith KR & Specter AC (2014). The importance of the presence of a 5′-ribonucleotide and the contribution of the T1R1 + T1R3 heterodimer and an additional low-affinity receptor in the taste detection of L-glutamate as assessed psychophysically. *J Neurosci* **34**, 13234–13245.

Toyono T, Seta Y, Kataoka S, Harada H, Morotomi T, Kawano S, Shigemoto R & Toyoshima K (2002). Expression of the metabotropic glutamate receptor, mGluR4a, in the taste hairs of taste buds in rat gustatory papillae. *Arch Histol Cytol* **65**, 91–96.

Toyono T, Seta Y, Kataoka S, Kawano S, Shigemoto R & Toyoshima K (2003). Expression of metabotropic glutamate receptor group I in rat gustatory papillae. *Cell Tissue Res* **313**, 29–35.

Treesukosol Y & Spector AC (2012). Orosensory detection of sucrose, maltose, and glucose is severely impaired in mice lacking T1R2 or T1R3, but polycose sensitivity remains relatively normal. *Am J Physiol Regul Integr Comp Physiol* **303**, R218–R235.

Yamaguchi S (1967). The synergistic taste effect of monosodium glutamate and disodium 5′-inosinate. *J Food Sci* **32**, 473–478.

Yang H, Wanner IB, Roper SD & Chaudhari N (1999). An optimized method for in situ hybridization with signal amplification that allows the detection of rare mRNAs. *J Histochem Cytochem* **47**, 431–446.

Yasumatsu K, Katsukawa H, Sasamoto K & Ninomiya Y (2003). Recovery of amiloride-sensitive neural coding during regeneration of the gustatory nerve: behavioral–neural correlation of salt discrimination. *J Neurosci* **23**, 4362–4368.

Yasumatsu K, Ogiwara Y, Takai S, Yoshida R, Iwatsuki K, Torii K, Margolskee RF & Ninomiya Y (2012). Umami taste in mice uses multiple receptors and transduction pathways. *J Physiol* **590**, 1155–1170.

Zhang F, Klebansky B, Fine RM, Xu H, Pronin A, Liu H, Tachdjian C, Li X (2008). Molecular mechanism for the umami taste synergism. *Proc Natl Acad Sci U S A* **105**, 20930–20934.

Zhao GQ, Zhang Y, Hoon MA, Chandrashekar J, Erlenbach I, Ryba NJ & Zuker CS (2003). The receptors for mammalian sweet and umami taste. *Cell* **115**, 255–266.

Additional information

Competing interests

The authors declare no competing interests.

Author contributions

Y.N. and K.Y designed and performed the research. K.Y. wrote the initial draft. K.Y. and T.M. performed recordings of taste responses and analysed data. Y.N., K.I., R.Y. H.U. and I.T. contributed to analyse the data and edited the draft. All authors read and approved the final version. All experiments were done in the Graduate School of Dental Sciences, Kyushu University.

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