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Astringency Is a Trigeminal Sensation That Involves the Activation of G Protein–Coupled Signaling by Phenolic Compounds

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Abstract

Astringency is an everyday sensory experience best described as a dry mouthfeel typically elicited by phenol-rich alimentary products like tea and wine. The neural correlates and cellular mechanisms of astringency perception are still not well understood. We explored taste and astringency perception in human subjects to study the contribution of the taste as well as of the trigeminal sensory system to astringency perception. Subjects with either a lesion or lidocaine anesthesia of the *Chorda tympani* taste nerve showed no impairment of astringency perception. Only anesthesia of both the lingual taste and trigeminal innervation by inferior alveolar nerve block led to a loss of astringency perception. In an in vitro model of trigeminal ganglion neurons of mice, we studied the cellular mechanisms of astringency perception. Primary mouse trigeminal ganglion neurons showed robust responses to 8 out of 19 monomeric phenolic astringent compounds and 8 polymeric red wine polyphenols in Ca^{2+} imaging experiments. The activating substances shared one or several galloyl moieties, whereas substances lacking the moiety did not or only weakly stimulate responses. The responses depended on Ca^{2+} influx and voltage-gated Ca^{2+} channels, but not on transient receptor potential channels. Responses to the phenolic compound epigallocatechin gallate as well as to a polymeric red wine polyphenol were inhibited by the Gαs inactivator suramin, the adenylate cyclase inhibitor SQ, and the cyclic nucleotide–gated channel inhibitor L-*cis*-diltiazem and displayed sensitivity to blockers of Ca^{2+}-activated Cl^- channels.

Key words: calcium imaging, capsaicin, chemesthesis, epigallocatechin gallate, hemiageusia, TRP channels

Introduction

Astringency is an everyday sensory experience best described as the sensation of oral dryness that is elicited by foodstuff with high polyphenol content, for example, unripe fruit, seeds, tea, cocoa, and red wine. Typical plant astringents are low–molecular weight polyphenols, such as the green tea flavan-3-ol epigallocatechin gallate, as well as polymeric tannins. The latter can be subclassified into condensed and hydrolyzable tannins (Hagerman 2002). According to the descriptors for different qualities of astringency (Gawel et al. 2000), flavan-3-ols elicit a prototypic rough and puckering astringency, whereas flavanon glycosides generate a soft and velvety astringent mouthfeel (Scharbert et al. 2004; Hufnagel and Hofmann 2008b).

Astringents activate the rodent *Chorda tympani* taste nerve together with fibers of the *N. glossopharyngeus* (Kawamura et al. 1969; Schiffman et al. 1992), suggesting that astringency is a taste sensation. However, in psychophysical studies, astringents could be perceived on nontaste oral tissues (Breslin et al. 1993; Lim and Lawless 2005), suggesting it is a somatosensation. Furthermore, perceived astringency increases with repetitive sampling (Guinard et al. 1986; Lyman and Green 1990; des Gachons et al. 2012), a typical feature of trigeminal, but not taste, sensations. At present, it is postulated that the precipitation of salivary proline-rich proteins by polyphenols reduces the lubrication of oral surfaces (Luck et al. 1994; Feldman et al. 1999; Jöbstl et al. 2004). As a consequence, mechanosensors of somatosensory nerves will be activated leading to the sensation of oral dryness (Lyman and Green 1990). However, the psychophysical function of a given astringent does not necessarily match its potency to precipitate protein and some astringents do not precipitate protein at all (Schwarz and Hofmann 2008; Ferrer-Gallego et al. 2012). In psychophysical studies, the time courses of protein binding and astringency perception (Kallithraka et al. 2001) or of salivary flow and astringency perception were not correlated (Guinard et al. 1997). It can be speculated that astringency is not purely if at all mediated by mechanosensors but might involve chemosensory detection mechanisms. Chemosensors of the taste and somatosensory systems are equipped with a plethora of specialized receptors apt to detect a wide range of chemically diverse stimuli (Chandrashekar et al. 2006; Ramsey et al. 2006; Damann et al. 2008; Chaudhari and Roper 2010). Several astringent phenols activate bitter taste receptors (Soares et al. 2013) explaining the bitterness of many astringents. Another study described the activation of transient receptor potential (TRP) channel A1 by epigallocatechin gallate (EGCG) (Kurogi et al. 2012) raising the possibility that trigeminal neurons expressing TRPA1 detect astringents. This work sheds light on the neural correlate and cellular basis of astringency perception. In psychophysical experiments, we gathered evidence showing that astringency is a trigeminal sensation. In an animal model, we studied the possible mechanisms of trigeminal neuron activation by astringents using primary trigeminal ganglion (TG) neurons of mice. Stimulation with astringents that display one or several galloyl moieties stimulated Ca^{2+} responses in these neurons. Furthermore, we identified a G protein–coupled signaling cascade activated by 2 structurally different astringents.

Materials and methods

Ethics statement

All aspects of the psychophysical study were performed with the informed consent of each individual and in accordance with The 1975 Declaration of Helsinki. The study was approved by the Institutional Review Board of the University of Florida (USA) or the Ethics Board of the Medial Faculty of the Ruhr-University Bochum (Germany). All experiments involving animals were carried out in accordance with the European Union Community Council guidelines and all measures were taken to minimize animal suffering.

Human subjects

The frontal aspect of the tongue is innervated by the *Chorda tympani* nerve (CTN), a branch of cranial nerve (CN) VII (Scott 2005), and the lingual nerve, a branch of CN V (Biedenbach and Chan 1971; Trulsson and Essick 1997). Twelve subjects (49 ± 15 years) with unilateral transection of the CTN, CN VII (Figure 1A), due to middle ear surgery participated in tests of taste and trigeminal perception 56 ± 19 hours postsurgery (hps). As a control group, 5 subjects (48 ± 22 years) without lesion of the CTN after middle ear surgery (nonlesioning surgery) were tested at 48 ± 17 hps. The medically necessary surgeries were performed at the St. Elisabeth Hospital, Bochum, Germany, by trained medical staff. In the lidocaine injection study, we tested 9 control subjects, 6 subjects after bilateral anesthesia of their CTNs by middle ear injection, and 4 subjects after bilateral anesthesia of their CTNs together with the lingual branch of the trigeminal nerve (LN, CN V) by inferior alveolar nerve block (Figure 1A). Lidocaine anesthesia was performed by trained medical staff at the University of Florida, Gainesville, USA, and with the written consent of each individual. Using a questionnaire, subjects were trained to rate sensory experiences according to the general labeled magnitude scale (gLMS) as described previously (Schöbel, Kyereme, et al. 2012). Subjects were familiarized with the sensory quality "astringent" by explanation using the terms "dry mouthfeel," "roughness," "do not confuse with bitter," and "long-lasting" as well as by sampling 20 mL astringent grape seed extract solution (see below), which is an astringent but nonbitter stimulus.

Test solutions

For psychophysical tests, 1 M sodium chloride (NaCl, salty), 1 M sucrose (sweet), 0.032 M citric acid (sour), and 1 mM quinine hydrochloride (bitter) were prepared in distilled water and kept in inert glass containers at 4 °C until use. For grape seed solution, 200 mg grape seed extract (Country Life) was mixed with 250 mL distilled water, filtered to remove solid

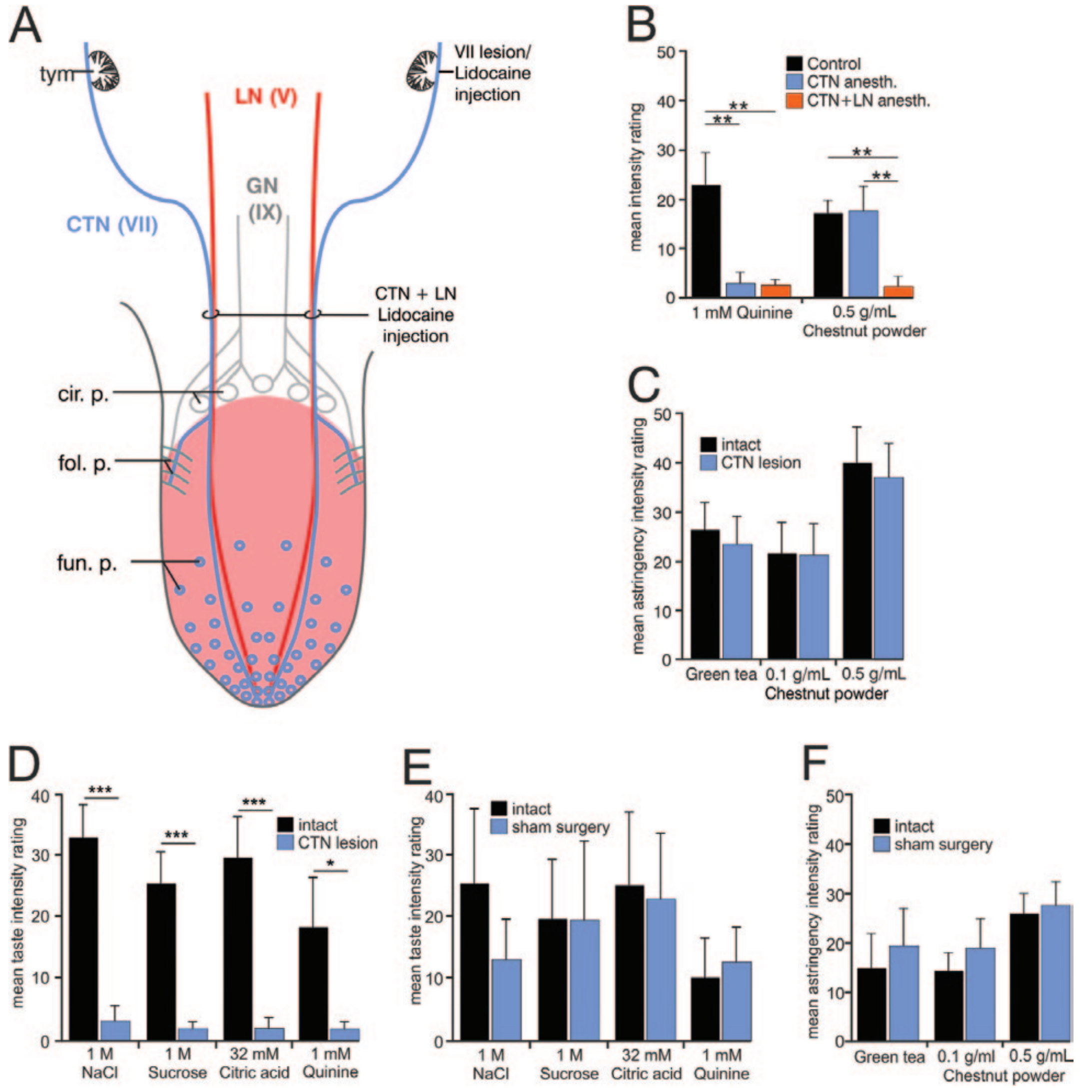

Figure 1 Psychophysical studies on human astringency perception. (**A**) Scheme of the lingual innervation with the surgery and injection sites relevant for the present study. tym = tympanum; CTN = *Chorda tympani* (CN VII); cir. p. = circumvallate papillae; fol. p = foliate papillae; fun. p. = fungiform papillae; LN = N. lingualis, CN V; GN = N. glossopharyngeus (CN IX). (**B**) Mean intensity ratings for bitter taste and astringency by 9 control subjects, 6 subjects with bilateral CTN lidocaine anesthesia (middle ear injection), and 4 subjects with bilateral CTN + LN lidocaine anesthesia (inferior alveolar nerve block). (**C**) Mean astringency intensity ratings by 12 subjects with documented unilateral lesion of the CTN. (**D**) Mean taste intensity ratings by 12 subjects with documented unilateral lesion of the CTN. (**E**) Mean taste intensity ratings by 5 subjects after nonlesioning middle ear surgery. (**F**) Mean astringency intensity ratings by 5 subjects after nonlesioning middle ear surgery. $***P \leq 0.001$, $**P \leq 0.01$, and $*P \leq 0.05$ (*U* test).

particles, and kept in inert glass at 4 °C until use. Green tea infusion was prepared by incubation of 5g Sencha tea in 20mL boiling water for 10min, followed by centrifugation (1000rpm, 5min). The supernatant was stored at −20 °C until use. European chestnut (*Castanea sativa*) powder (Presque Isle Wine Cellars) was prepared at 0.1 and 0.5g/mL in distilled water. Solutions were centrifuged at 1000rpm for 10min and the supernatant was stored at −20 °C until use. Prior to use, samples were allowed to equilibrate to room temperature.

Psychophysical tests

Test solutions were applied to the tongue with sterile cotton swabs. For tests of taste perception, the fungiform papillae on both sides of the tongue were briefly touched with the cotton swab, one side at a trial. A gLMS was always visible to the subjects. Subjects were asked to decide on their taste intensity rating prior to taking back the tongue into the mouth and then verbalize their decision. Thereby, a mixing of the taste stimulus with saliva and stimulus spreading onto other taste-receptive areas happened only after the subjects had decided on their intensity ratings. Following the taste stimuli, astringent stimuli were applied to the fungiform papillae by rolling the cotton swab along a 2-cm strip about 0.5cm beside the midline of the tongue. The astringent stimuli we used, albeit quite intense, could only hardly be perceived as astringent without any tongue movement. Subjects were thus asked to move the tongue back and forth once against the hard palate and then rate the perceived astringency on the tongue. After each trial, subjects were advised to rinse with water thoroughly. Whenever necessary, it was waited for 2–5 min for residual astringency to wear off before another astringent stimulus was applied.

Animals

CD1 mice were obtained from Charles River. Animals were kept in a 12:12 h light:dark cycle and offered regular laboratory chow and water ad libitum.

Preparation of TG neurons of mice

Mice aged postnatal day (P) 0–5 were decapitated, the ganglia dissected, collected in ice cold Leibovitz medium (L15, Invitrogen), transferred to essential medium (minimum essential medium, Invitrogen) containing 0.025% collagenase (type IA, Sigma), and then incubated for 45 min (37 °C, 6% CO_2). The tissue was then triturated with fire polished glass pipettes. The suspension was centrifuged for 5 min at 1000 rpm, resuspended in Dulbecco's modified eagle medium F-12 (DMEM/F-12, GlutaMAX, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 100 U/mL penicillin and 100 µg/mL streptomycin (P/S), filtered (70 µm cell sieve, Falcon), and plated in 50 µL drops on poly-L-lysine (Sigma)-coated glass cover slips. After 1 h, 2 mL DMEM/F-12 (+FBS/+P/S) were added and cultures were used for experiments within 1–3 days in vitro. Experiments on TG neurons were performed on at least 3 biological replicates.

Chemicals

Chemicals were prepared as stocks in distilled water, EtOH, or dimethyl sulfoxide (DMSO) and diluted to their final concentration with standard assay buffer (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES]), giving a maximal solvent concentration of 0.1%. Caffeic acid-L-3,4-dihydroxyphenylalanine (DOPA) and caffeic acid-L-phenylalanine were synthesized as reported (Stark and Hofmann 2005). Rutin was isolated from green tea (Scharbert et al. 2004). Myricetin was provided by Senn Chemicals. Caftaric acid, catechin gallate (CG), 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid disodium salt (DIDS), epicatechin gallate (ECG), ferulic acid, gallic acid, gallocatechin gallate (GCG), kaempferol, luteolin, menthol (ME), mibefradil, niflumic acid (NFA), protocatechuic acid, quercetin, quercetin 3-D-galactoside, ruthenium red, tannic acid, and U-73122 were purchased from Sigma–Aldrich. Capsaicin (CAP) and SQ22536 were obtained from Calbiochem, ω-conotoxin MVIIC and nimodipine from Tocris Bioscience, EGCG and thapsigargin from Enzo Life Sciences, Inc., L-*cis*-diltiazem from Biomol, mustard oil (MO) from Fluka, and quinine HCl (food grade) from Alfa Chem.

Isolation of high–molecular weight polymeric polyphenols from red wine

The isolation and characterization of the polymeric red wine polyphenols have been described recently (Wollmann and Hofmann 2013). In brief, the polymeric red wine fraction was isolated from red wine by ultrafiltration using a VIVACELL 250 static gas pressure filtration system equipped with a 5-kDa molecular weight cutoff VIVACELL 250 5,000 MWCO PES membrane (Vivascience). The retentate was lyophilized to afford the high–molecular weight polymers (HMW fraction; >5 kDa) in a yield of 4.5 g/L. An aliquot (800 mg) of the polymers was dissolved in methanol/water (20/80, v/v; pH 4.5) acidified with traces of formic acid and separated on Sephadex LH 20 (GE Healthcare) using a 100 × 5 cm XK50/100 glass column (GE Healthcare Bio-Science AB) and methanol/water (20/80, v/v; pH 4.5) as the effluent. Chromatography (flow rate 1.8 mL/min) was performed with the methanol/water mixtures 20/80 (v/v; 7 h), 40/60 (v/v; 16 h), 60/40 (v/v; 7 h), 80/20 (v/v; 16 h), 100/0 (v/v; 16 h), followed by a mixture of acetone/water (70/30, v/v; pH 4.5) for 16 h. The effluent was collected in 8 fractions, separated from solvent in vacuum to afford the subfractions f1 (1.6 g/L), f2 (0.3 g/L), f3 (0.4 g/L), f4 (0.5 g/L), f5 (0.7 g/L), f6 (0.2 g/L), f7 (0.4 g/L), and f8 (0.3 g/L) as amorphous powders in the yields given in parenthesis (calculated as concentration in wine). According to literature protocols (Thompson et al. 1972; Preys et al. 2004), the mean degree of flavan-3-ol polymerization (mDP) and the percentage of galloylation were determined in all fractions and are given in parenthesis: f1 (not detected/not detected), f2 (8.5/11.6), f3 (6.0/8.4), f4 (8.2/12.0), f5 (7.3/13.7), f6 (5.8/9.1), f7 (9.3/13.2), and f8 (10.1/17.3).

Ca^{2+} imaging

Cells were incubated with 3 mM Fura-2/AM (Invitrogen) in cell culture media at 37 °C and 6% CO_2 for 1 h. Glass slides were mounted on an inverted microscope (Axiovert 200, Zeiss) equipped with a fluorescence-optimized 20X Zeiss UplanApo (×20/0.75) objective. Cells were excited intermittently at 340 and 380 nm (Lambda DG4, Sutter Instrument Company, connected to a Uniblitz Vmm-D1 shutter driver and a Voltakraft condensor) at 1 Hz. Emission at 510 nm was detected by a Zeiss Axiocam MRM charge-coupled-device camera. Imaging data were acquired by the Slide-Book software (3I-Imaging). Changes of Ca^{2+} were measured as the ratio of emission at 510 nm for both excitation wavelengths (f_{340}/f_{380}). Substances were applied by a custom-made, pressure-driven 7-in-1 application system at a flow rate of 500 µL/min. Viability and neuronal phenotype were tested by stimulation with depolarizing buffer (100 mM NaCl, 45 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES). The Ca^{2+}-free buffer contained 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5 mM ethylene glycol tetraacetic acid (EGTA), and 10 mM HEPES. Solutions were adjusted to pH 7.4 (NaOH/HCl) and 310 mosmol/L (glucose).

Statistical tests

Baseline Ca^{2+} levels (mean of 10 time points prior to stimulation) and response amplitudes were calculated using Excel 2010 (Microsoft Corp.). The maximum response amplitude had to exceed the cell's baseline Ca^{2+} level plus 4 times the baseline's standard deviation to be counted as a response (4 Sigma criterion). Dose–response curves were fitted with IgorPro (Wavemetrics). Origin Pro (Systat Software Inc.) and SPSS Statistics 20 (IBM) were used for statistical analysis. Normal distribution ($P \geq 0.05$) was tested by Kolmogorov–Smirnov test. For normally distributed independent and dependent data sets, significance was tested by *t*-test. Nonnormally distributed and/or small data sets were analyzed by *U* test (Mann–Whitney) for independent or Wilcoxon test for dependent samples. Percentages were compared by Fisher's Exact test. All results in figures are presented as mean ± SEM.

Results

The trigeminal nerve detects lingual astringency

We performed 2 sets of psychophysical tests to examine the respective roles of the taste and trigeminal sensory systems in astringency perception. As a first approach, subjects received bilateral lidocaine anesthesia of either their CTN (middle ear injection) or their CTN + lingual branch of the trigeminal nerve (LN) (inferior alveolar nerve block) prior to the tests (Figure 1A). Subjects then rated the perceived taste intensity of bitter quinine solution (1 mM) or astringency of astringent chestnut powder solution (0.5 g/mL) according to the gLMS. In comparison to controls, subjects who underwent either type of lidocaine anesthesia gave lower intensity ratings for bitter taste than nonanesthetized subjects ($n = 9$ vs. $n = 6$ vs. $n = 9$, $P \leq 0.01$ [*U* test], Figure 1B). However, the intensity ratings for astringent chestnut solution were the same for the control and CTN anesthesia group. Only after CTN + LN lidocaine anesthesia, astringency perception by the subjects was strongly impaired ($P \leq 0.01$ [*U* test], Figure 1B). As a second approach, we evaluated taste and astringency perception in 12 human subjects with unilateral *Chorda tympani* taste nerve lesion (CTN, Figure 1A). Taste and astringent solutions were applied to the fungiform papillae on either the left or the right tip of the tongue and subjects rated the sensation according to a gLMS. The mean intensity ratings for the astringency of green tea and for 2 concentrations of chestnut powder solution were nearly identical on both hemitongues after unilateral CTN lesion (Figure 1C). However, subjects showed a near-complete loss of taste sensitivity for all tested taste qualities on the denervated hemitongue (Figure 1D). Subjects who underwent middle ear surgery without transection of the CTN showed no impairment of taste perception on the hemitongue ipsilateral to the surgical intervention (Figure 1E). Nonlesioning middle ear surgery also did not affect astringency perception on the hemitongue ipsilateral to the lesion (Figure 1F).

Taken together, astringency perception was unaffected by a loss of taste nerve function resulting from nerve lesion or lidocaine anesthesia but impaired by anesthesia of the lingual trigeminal innervation after inferior alveolar nerve block.

Astringent plant phenols activate TG neurons in vitro

We challenged isolated TG neurons of mice with plant-derived phenolic compounds. These compounds were isolated from tea, cocoa, and red wine and identified as astringents by sensory-guided fractionation procedures (Table 1). We applied all substances thrice to the cells for 20 s with an interval of 2 min during continuous monitoring of intracellular Ca^{2+} levels. Ferulic acid, caftaric acid, caffeic acid-L-DOPA, caffeic acid-L-phenylalanine, kaempferol, quercetin, quercetin 3-D-galactoside, rutin, and luteolin did not induce Ca^{2+} signals in TG neurons. We only rarely observed responses to protocatechuic acid or catechin (1.7% and 6.8% of all TG neurons, respectively). However, gallic acid, GCG, ECG, epigallocatechin (EGC), EGCG, myricetin, and tannic acid frequently elicited responses in TG neurons (12.1–65%, Table 1). Responses were typically initiated within a few seconds (≤5 s) after stimulation onset and characterized by a sharp rise of intracellular Ca^{2+} levels. In some TG neurons, we observed delayed responses that occurred within ≤10 s after stimulus onset and that occasionally occurred directly after or within a few seconds after stimulus offset. In most cells, Ca^{2+} levels reached their maximum 1–2 s after response onset. Intracellular Ca^{2+} levels declined in the presence of the stimulus (Figure 2A). However, we also observed responses characterized by an initial weak increase of intracellular Ca^{2+} followed by a sudden sharp Ca^{2+} rise (see Figure 6A, light gray trace). Interestingly, we observed that the presence of one or more galloyl moieties (galloylation) was highly correlated with the activation of TG neurons by a given astringent. Among 11 nongalloylated astringents, 2 stimulated responses in TG neurons in contrast to all of the 9 galloylated astringents ($P \leq 0.001$ [Fisher's Exact test], Table 1). Next, we compared the frequency of responses induced by protocatechuic acid and gallic acid. Both molecules are structurally identical except for 1 hydroxyl group that gives gallic acid a complete galloyl moiety. Interestingly, of all TG neurons tested, 21.7% responded to gallic acid and 1.7% to protocatechuic acid. However, the EC_{50} values of

Table 1 Characteristics of plant phenols tested on TG neurons

Substance	Classification	Source (literature)	*n* galloyl moieties	% responders	*n* cells	EC_{50} (µM)
Caffeic acid-L-DOPA	Hydrocinnamic acid	Cocoa (Stark et al. 2005)	0	0	49	
Caffeic acid-L-phenylalanine	Hydrocinnamic acid	Cocoa (Stark et al. 2005)	0	0	54	
Caftaric acid	Hydrocinnamic acid	Red wine (Hufnagel and Hofmann 2008a, 2008b)	0	0	56	
Catechin	Flavanol	Red wine (Hufnagel and Hofmann 2008a, 2008b) Cocoa (Stark et al. 2005) Tea (Scharbert et al. 2004)	0	6.8	59	
CG	Flavanol	Red wine (Hufnagel and Hofmann 2008a, 2008b) Tea (Scharbert et al. 2004)	1	23.3	99	
ECG	Flavanol	Tea (Scharbert et al. 2004)	1	12.1	140	
EGC	Flavanol	Tea (Scharbert et al. 2004)	1	44.4	144	>800
EGCG	Flavanol	Tea (Scharbert et al. 2004)	2	25.4	232	412 ± 36
Ferulic acid	Hydrocinnamic acid	Red wine (Hufnagel and Hofmann 2008a, 2008b) Cocoa (Stark et al. 2005)	0	0	77	
Gallic acid	Phenolic acid	Red wine (Hufnagel and Hofmann 2008a, 2008b) Tea (Scharbert et al. 2004)	1	21.7	115	
GCG	Flavanol	Tea (Scharbert et al., 2004)	2	65	262	
Kaempferol	Flavanone	Red wine (Sáenz-Navajas et al. 2010) Onion (Slimestad et al. 2007)	0	0	67	
Luteolin	Flavon	Cocoa (Stark et al. 2005)	0	0	61	
Myricetin	Flavanone	Red wine (Sáenz-Navajas et al. 2010; Vitrac et al. 2001) Onion (Slimestad et al. 2007)	1	40.6	155	85 ± 1
Protocatechuic acid	Phenolic acid	Red wine (Hufnagel and Hofmann 2008a, 2008b)	0	1.7	115	
Quercetin	Flavanone	Red wine (Hufnagel and Hofmann 2008a, 2008b) Tea (Scharbert et al. 2004)	0	0	96	
Quercetin 3-D-galactoside	Flavanone	Red wine (Hufnagel and Hofmann 2008a, 2008b) Tea (Scharbert et al. 2004)	0	0	54	
Rutin	Flavanone	Red wine (Hufnagel and Hofmann 2008a, 2008b) Tea (Scharbert et al. 2004) Red currant (Schwarz and Hofmann 2007)	0	0	225	
Tannic acid	Condensed tannin	Red wine (Hufnagel and Hofmann 2008a, 2008b)	5	52.6	95	102 ± 22

myricetin (1 galloyl moiety, 85 ± 1 µM), EGC (1 galloyl moiety, >800 µM), EGCG (2 galloyl moieties, 412 ± 36 µM), and tannic acid (5 galloyl moieties, 102 ± 22 µM) did not correlate with the number of galloyl moieties for galloylated astringents. Similarly, we did not observe a correlation between the number of galloyl moieties and the response frequencies (Figure 2B and Table 1). Figure 3 shows the spectrum of all tested phenolic astringents that activated or did not activate TG neurons in our Ca^{2+} imaging experiments. In summary, of the 19 astringents tested here, 8 robustly activated large proportions of TG neurons. The presence of at least 1 galloyl motif seems to be crucial for the activation of TG neurons.

EGCG does not activate TRP channels

About 25% of all TG neurons responded to EGCG in the initial experiments. EGCG is the main astringent compound of green tea (~30% of the dry mass), thus accounting for

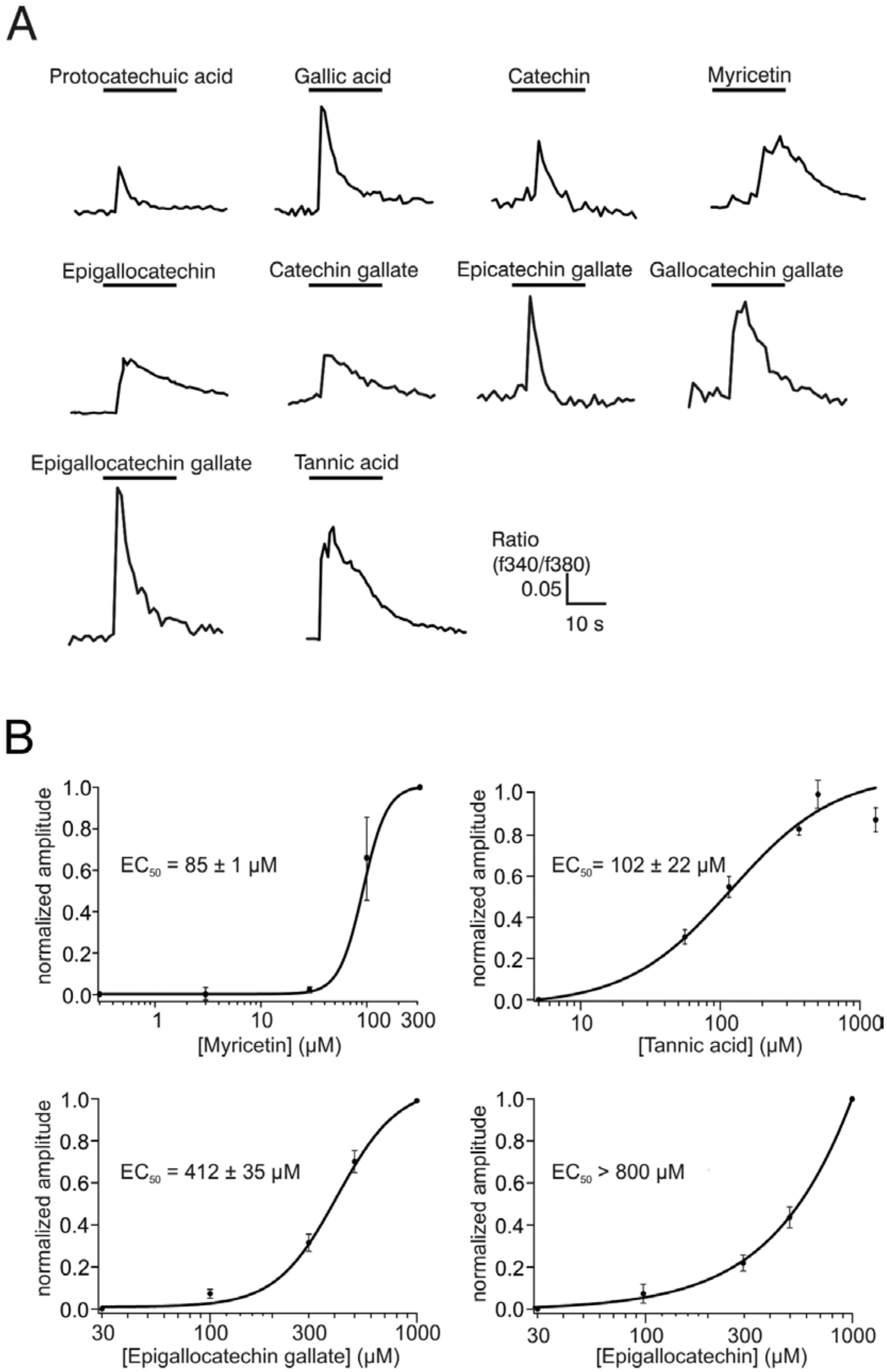

Figure 2 Activation of TG neurons by plant astringents. (**A**) Representative Ca^{2+} imaging recordings of TG neurons stimulated with phenolic astringents at a concentration of 500 µM (except myricetin that was maximally soluble to 300 µM). Horizontal bars indicate application times. (**B**) Dose–response curves for chosen flavonoids obtained in Ca^{2+} imaging experiments on TG neurons. Myricetin (1 galloyl moiety, *n* = 42), tannic acid (5 galloyl moieties, *n* = 116), EGCG (2 galloyl moieties, *n* = 132), and EGC (1 galloyl moiety, *n* = 98).

most of the tea's astringency. Because of EGCG's relevance as a food astringent and the existence of a considerable body of research on its effects in different cellular models, we chose it as a model substance for further experiments. We applied a saturating concentration of 1 mM EGCG to TG neurons that we consecutively also stimulated with typical trigeminal stimuli. In more detail, we used 3.3 µM CAP to identify TRP channel V1–positive sensors of noxious heat (Caterina et al. 1997), 300 µM ME for TRPM8-positive cold sensors (McKemy et al. 2002; Peier et al.

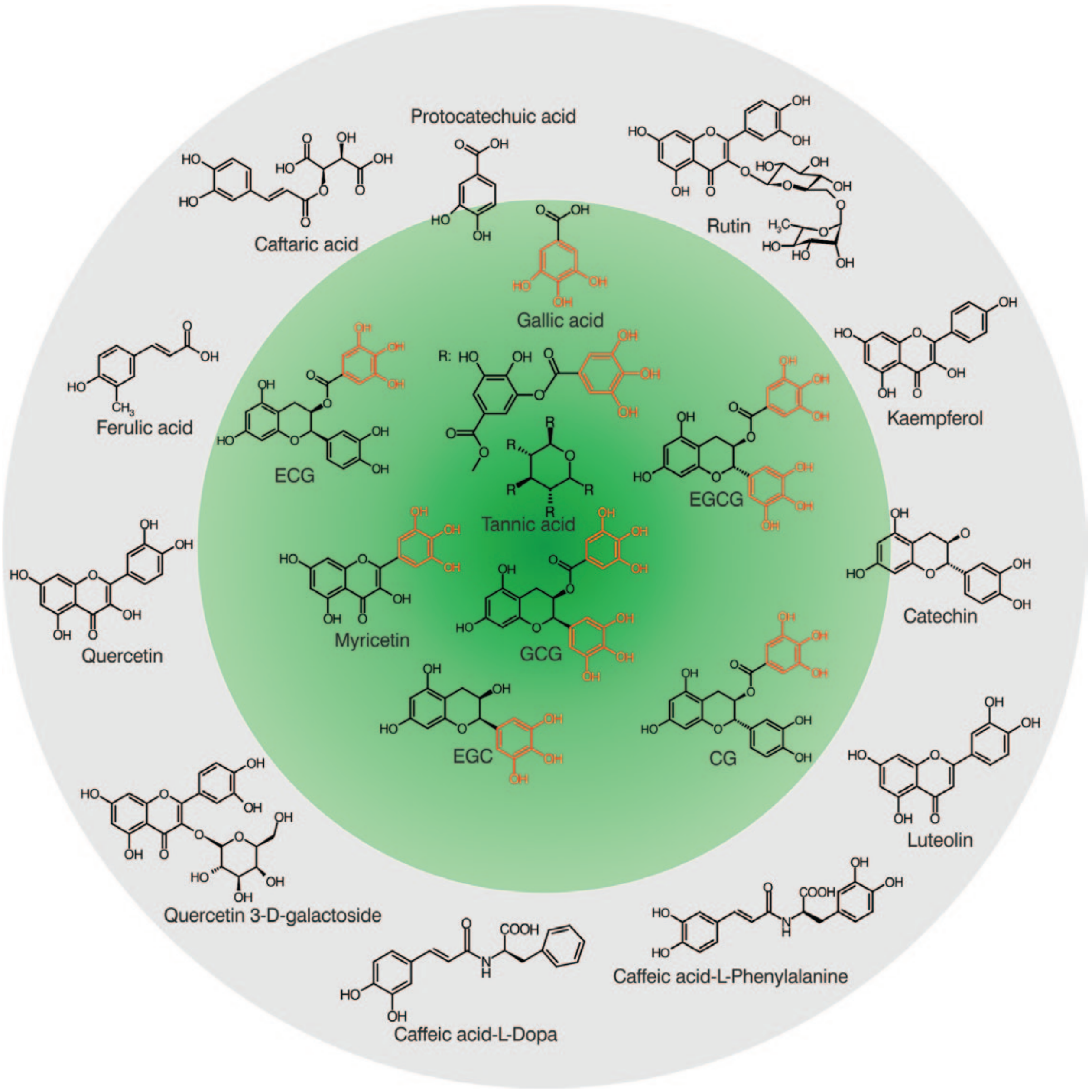

Figure 3 Molecular receptive field of phenolic plant astringents on TG neurons. Gray area: nonactivating substances; margin: weakly activating substances; green area: strongly activating substances. Galloyl moieties are highlighted in red.

2002), 50 µM MO for TRPA1-positive sensors of irritants (Story et al. 2003; Bandell et al. 2004; Jordt et al. 2004), and hypoosmolaric assay buffer (220 mosmol, HYPO) to identify TRPV4-positive mechanosensitive neurons (Liedtke et al. 2000; Strotmann et al. 2000). We tested all substances on each of the total 301 TG neurons (Figure 4A). The observed response frequencies upon challenge with CAP, ME, and MO were in accordance with data on dorsal root ganglion neurons of CD1 mice of the same age (Hjerling-Leffler et al. 2007). We frequently observed TG neurons that responded to several of the test stimuli (Figure 4A,C). Of all EGCG-sensitive neurons, 21.8% did not respond to any other stimulus, 9.1% to ME, 45.5% to HYPO, 9.1% to MO, and 60% to CAP (Figure 4C). The interaction between EGCG and CAP was significant ($P \leq 0.05$). In other terms, there is a higher likeliness for CAP-sensitive TG neurons to also show EGCG sensitivity. However, 46% of the EGCG responders were insensitive to CAP (Figure 4C). To test whether EGCG activate TG neurons through the activation of TRP channels, we challenged the cells with EGCG in the presence of the unspecific TRP channel blocker ruthenium red. Ruthenium red (10 µM) completely inhibited responses to 500 nM CAP but had no effect on responses to 250 µM EGCG (Figure 4D).

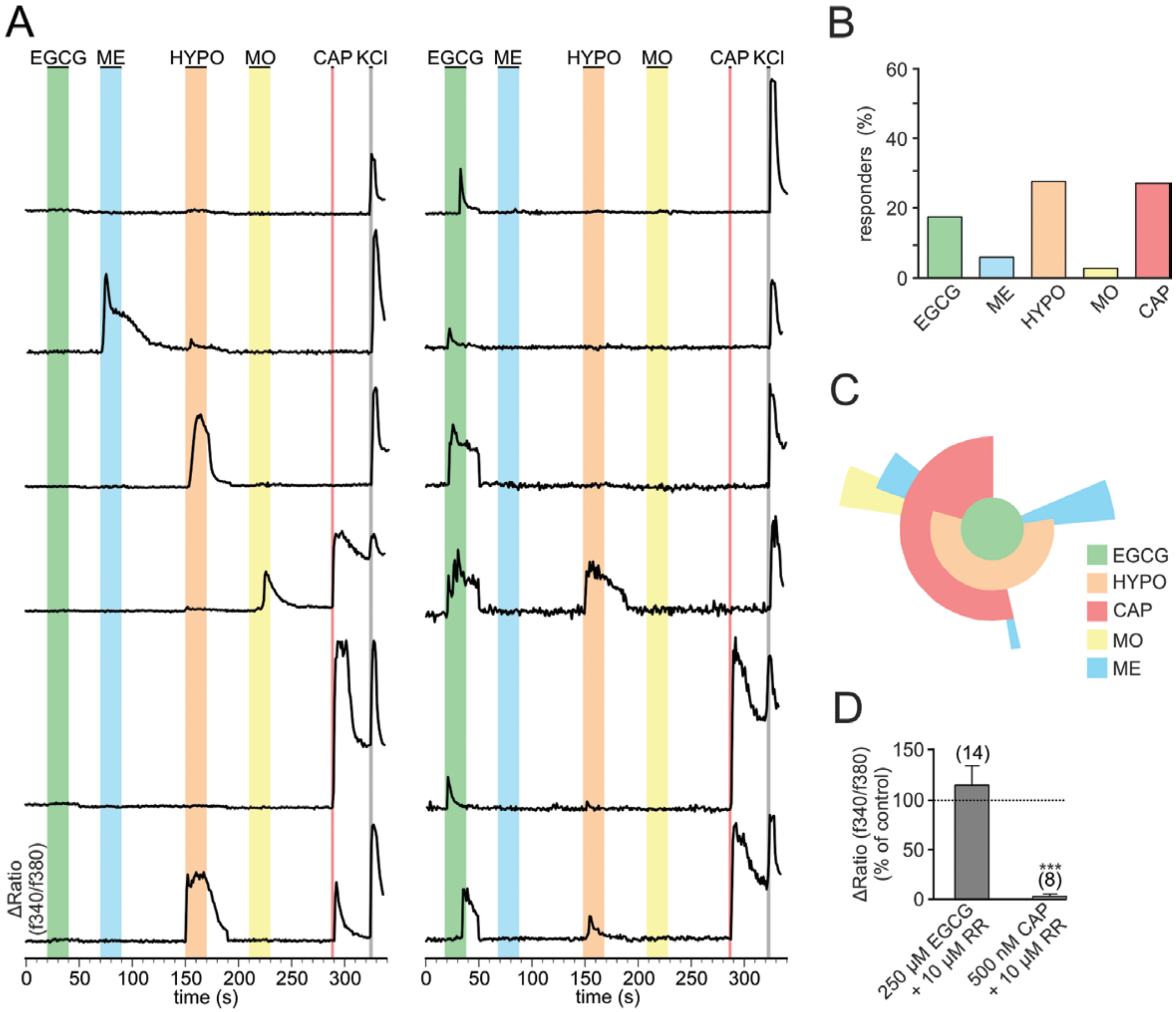

Figure 4 Involvement of TRP channels in EGCG detection by TG neurons. (**A**) Typical Ca^{2+} imaging recordings of TG neurons stimulated with 1 mM EGCG and 300 µM ME, HYPO (220 mosmol/L), 50 µM MO, and 3.3 µM CAP (*n* = 301). Colored vertical bars indicate application times. (**B**) Percentages of all viable TG neurons responsive to EGCG, ME, HYPO, MO, and CAP. As many TG neurons were multiply responsive, added percentages exceed 100%. (**C**) Diagram showing the coresponsiveness of all EGCG-sensitive TG neurons to one or several of the typical trigeminal stimuli ME, HYPO, MO, and CAP. More than half of all EGCG-sensitive TG neurons (green inner circle) were also sensitive to HYPO (light orange area). Among those EGCG and HYPO responsive neurons, about two-thirds were also sensitive to CAP (red area). Of the neurons sensitive to EGCG, HYPO, and CAP, small fractions were also activated by ME or MO (blue and yellow areas, respectively). (**D**) Mean response amplitude of TG neurons to 250 µM EGCG or 500 nM CAP in the presence of 10 µM ruthenium red (*n* = 27).

Together, these experiments suggest that EGCG sensitivity is distributed across different subpopulations of trigeminal sensory neurons. Among all subpopulations tested, EGCG activated CAP-sensitive neurons with a higher probability. The unspecific TRP channel antagonist ruthenium red did not inhibit responses to EGCG.

EGCG stimulates G protein–coupled signaling in TG neurons

We investigated the signal transduction mechanisms that underlie the activation of TG neurons by EGCG. Under control conditions, response amplitudes to 250 µM EGCG slightly sensitized repetitive upon application at a 2-min interstimulus interval (Figure 5A). Responses to the same concentration of EGCG were completely abolished in EGTA-buffered Ca^{2+}-free buffer (Figure 5A). Similarly, blocking the function of voltage-gated Ca^{2+} channels (VGCCs) by a combination of 10 µM nimodipine, 10 µM mibefradil, and 1 µM ω-conotoxin MVIIC greatly diminished responses to EGCG (Figure 5A). In contrast to that, prior depletion of endoplasmic Ca^{2+} stores with 10 µM thapsigargin did not diminish responses arguing against an involvement of intracellular Ca^{2+} sources (Figure 5A).

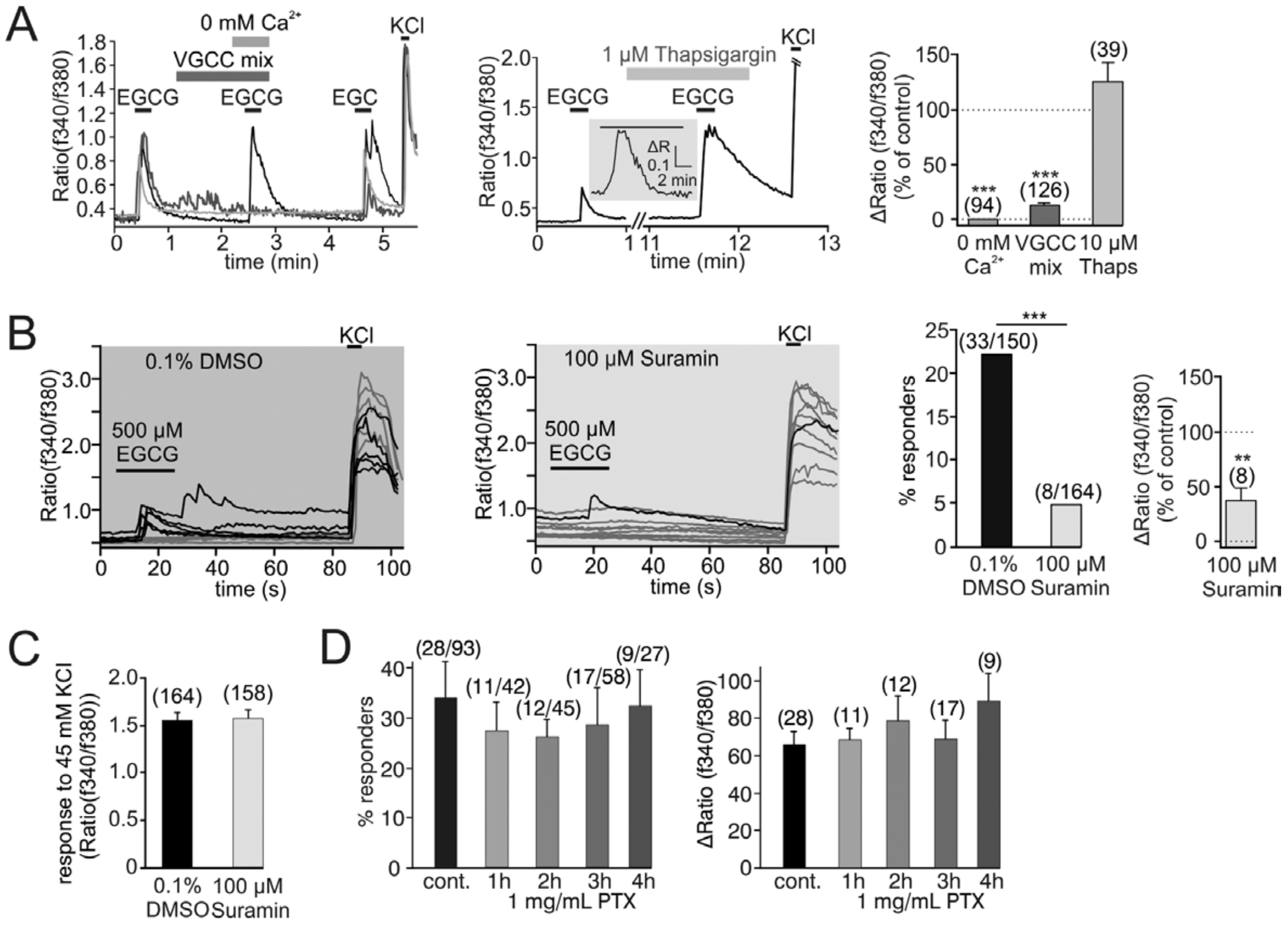

Figure 5 Investigation of signal transduction events that underlie the activation of TG neurons by EGCG. (**A**) Effects of Ca^{2+} buffering, coapplication of VGCC inhibitors, or preincubation with thapsigargin on the responses to 250 µM EGCG. Inlay in the thapsigargin example shows the intracellular Ca^{2+} dynamics following store depletion by 10 µM thapsigargin treatment. (**B**) Effects of DMSO or suramin preincubation on the responses to 500 µM EGCG. (**C**) Effects of DMSO or suramin preincubation on the responses to depolarizing buffer containing 45 mM KCl. (**D**) Effects of pertussis toxin (PTX) preincubation on the responses to 1 mM EGCG. Sample sizes are given in parentheses. $***P \leq 0.001$ and $**P \leq 0.01$.

G protein–coupled heptahelical receptors (7TM receptors) are required for chemosensory detection in olfactory sensory neurons (Buck and Axel 1991), taste receptor cells (Chandrashekar et al. 2006; Chaudhari and Roper 2010), and vomeronasal neurons (Halpern and Martínez-Marcos 2003). They couple to a plethora of G proteins. To test for an involvement of G protein–triggered pathways in the activation of TG neurons by EGCG, we incubated TG neurons for 60 min with 100 µM suramin to dissociate the receptor to G-protein coupling (Chung and Kermode 2005). After suramin treatment, 4.9% of the TG neurons responded to EGCG (0.1% DMSO control: 22%, $P \leq 0.001$) and the response amplitudes of the few responding cells were greatly diminished (Figure 5B). As a control, 100 µM suramin did not reduce the responses to a depolarizing assay buffer containing 45 mM KCl (Figure 5C). Responses to 1 mM EGCG were insensitive to 1 mg/mL of the Gαi/o blocker pertussis toxin (Figure 5D). Among the downstream targets of Gαs proteins are phospholipase C and adenylyl cyclase. In the presence of 50 µM of the phospholipase C inhibitor U-73122 (Klasen et al. 2012), responses to EGCG were not diminished. The adenylyl cyclase blocker SQ22536 (100 µM; Spehr et al. 2004) completely inhibited EGCG responses in 75% of the cells (Figure 6A). In the remaining 25% of EGCG responders, SQ22536 diminished responses to 53 ± 11% of controls (n = 6, Figure 6B). Responses to 45 mM KCl were not affected by the blocker (Figure 6A). Adenylyl cyclase generates cyclic adenosine monophosphate (cAMP) that can activate cyclic nucleotide-gated (CNG) channels. Lately, the olfactory CNG channel CNGA2 was identified in TG tissue by next-generation sequencing (Manteniotis et al. 2013) indicating the presence of a cAMP target channel in TG neurons. After preincubation with the CNG channel inhibitor L-*cis*-diltiazem at a concentration of 100 µM (Frings et al. 1992), 5% of the TG neurons showed responses to EGCG in comparison to 22% that responded after 0.1% DMSO treatment as a solvent control ($P \leq 0.001$,

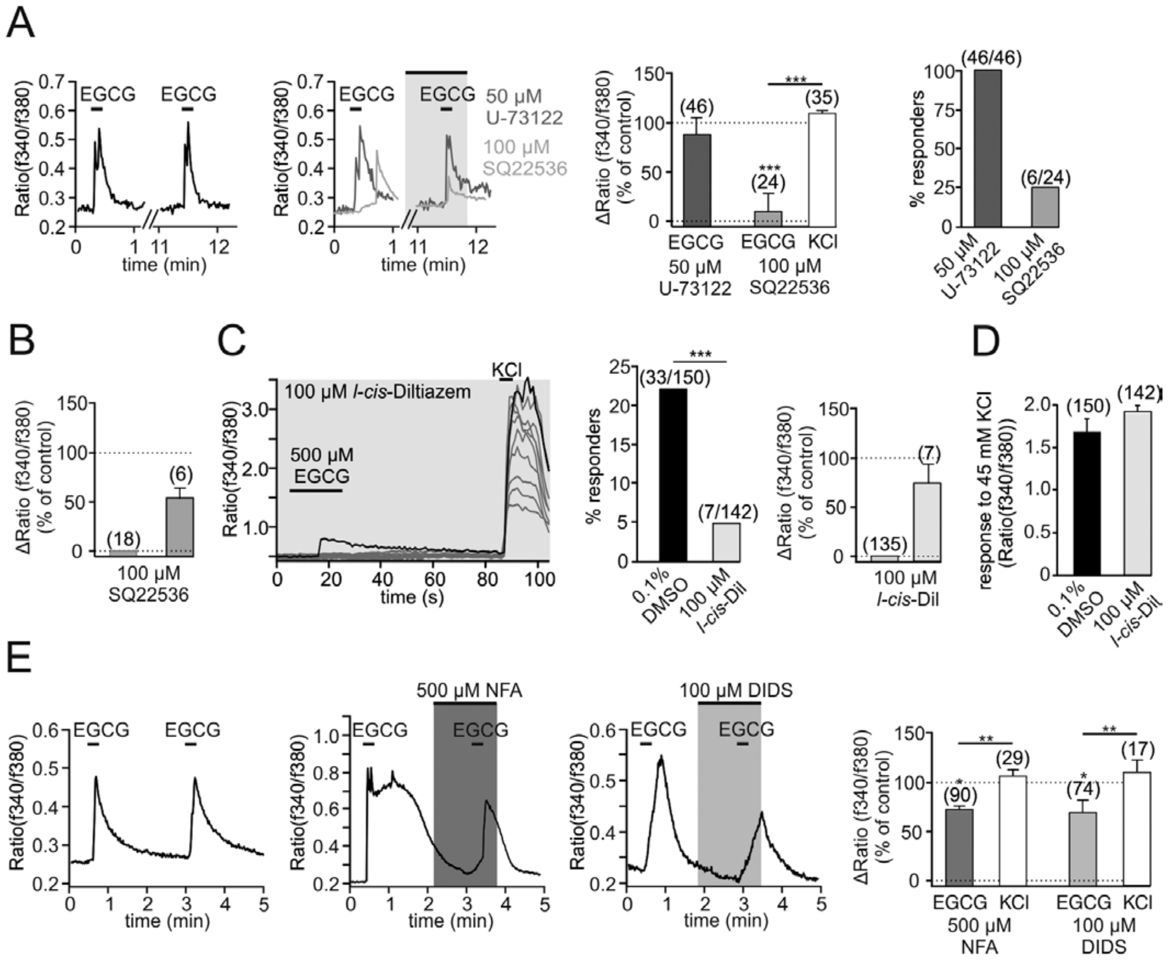

Figure 6 Investigation of second messenger–mediated events that underlie the activation of TG neurons by EGCG. (**A**) Effects of 50 µM U-73122 or 100 µM SQ22536 treatment on responses to 250 µM EGCG and of SQ22536 on responses to 45 mM KCl. (**B**) Quantification of the complete and partial block of responses to 250 µM EGCG in the presence of 100 µM SQ22536. (**C**) Effects of 100 µM L-*cis*-diltiazem preincubation on the responses to 500 µM EGCG. Note that responses were only partially inhibited in some TG neurons. (**D**) Effects of L-*cis*-diltiazem preincubation on the responses to depolarizing buffer containing 45 mM KCl. (**E**) Effects of coapplication of the CaCC blockers NFA or DIDS on the responses to 500 µM EGCG and 45 mM KCl. Sample sizes are given in parentheses. ****P* ≤ 0.001 and **P* ≤ 0.05.

Figure 6C). The response amplitudes of the cells that still responded in the L-*cis*-diltiazem condition were not significantly reduced (Figure 6C). At this concentration, L-*cis*-diltiazem did not diminish responses to depolarizing buffer (Figure 6D). Therefore, it is unlikely that off-target effects of the drug on voltage-gated channels described elsewhere (Ikeda et al. 1991; Hashimoto et al. 2000; Baumann et al. 2004; Leung et al. 2010) affected our experiments. Recently, we described active intracellular Cl^- accumulation and signal amplification by Cl^- efflux through Ca^{2+}-activated Cl^- channels (CaCCs) in TG neurons (Schöbel, Radtke, et al. 2012). We asked whether a Cl^--based mechanism might amplify responses also to EGCG. In the presence of the CaCC blockers NFA (500 µM) or DIDS (100 µM) (Schöbel, Radtke, et al. 2012), EGCG-induced responses of TG neurons were indeed diminished by nearly 30%, but both drugs had no effects on responses induced by 45 mM KCl (Figure 6E) or on response frequencies (not shown).

In conclusion, the results indicate that in most EGCG-sensitive TG neurons, EGCG activates a G protein–coupled pathway that recruits adenylate cyclase (AC), followed by the activation of CNG channels, and signal amplification through CaCCs. However, a fraction of EGCG-induced responses was not or only partially diminished by blockers of G proteins or a cAMP-mediated pathway indicating yet other transduction mechanisms.

Astringent polymeric red wine polyphenols activate TG neurons in vitro

Typical food astringents encompass monomeric phenols like the flavan-3-ol EGCG and polymeric tannins. A hallmark of red wine is its complex and well-balanced astringency that results mainly from polymeric HMW tannins (MW > 5 kDa) (Hufnagel and Hofmann 2008a). We asked whether TG neurons that bear the capacity of detecting monomeric

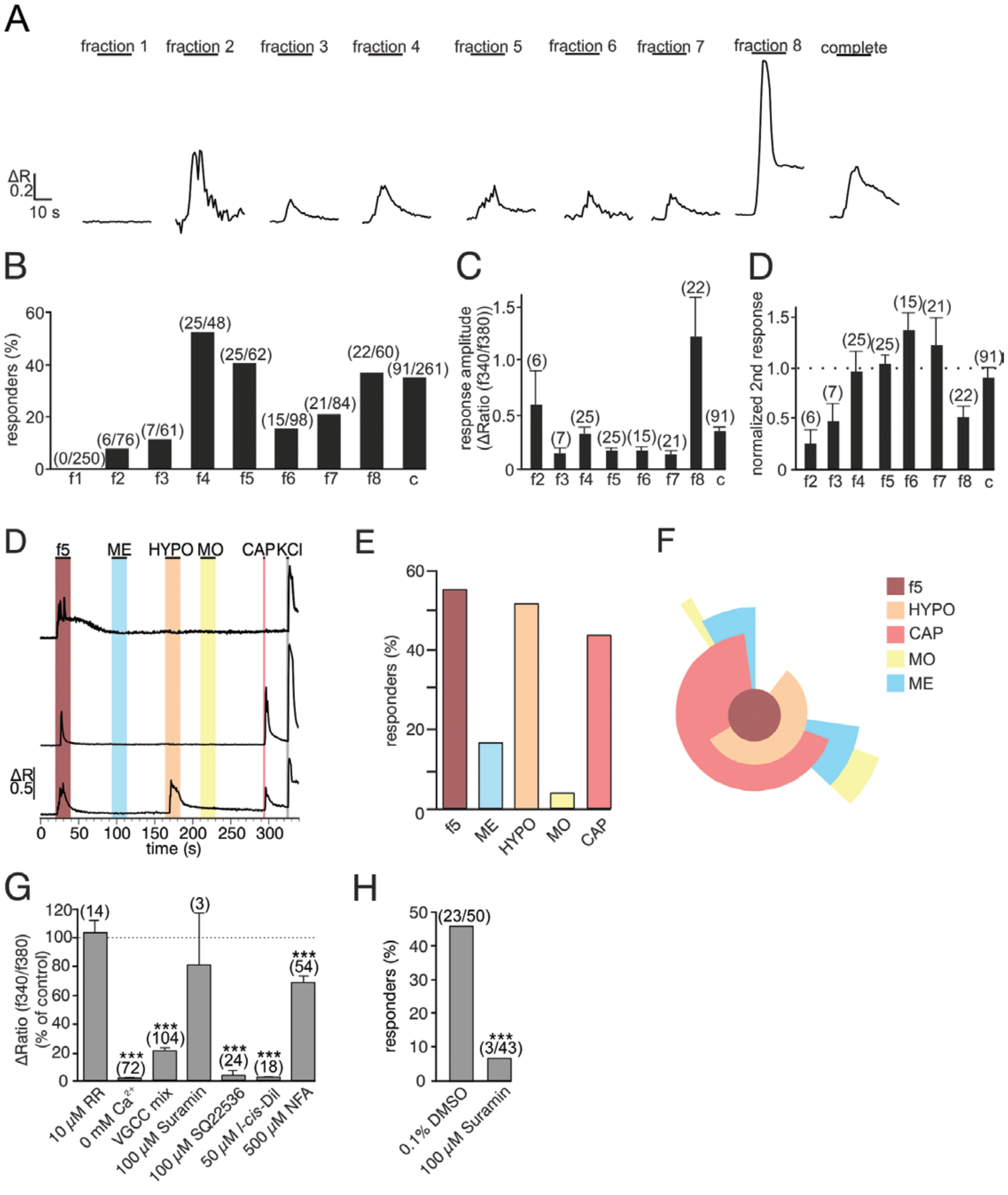

Figure 7 Functional analysis of responses of TG neurons to astringent wine polymers. (**A**) Representative Ca^{2+} responses to the stimulation with astringent wine polymers (100 µg/mL). Response frequencies (**B**) and mean response amplitudes (**C**) of TG neurons to different astringent wine polymers. (**D**) Normalized second response amplitudes to astringent wine polymers showing the sensitization or desensitization upon repetitive stimulation. (**E**) Representative Ca^{2+} imaging recordings of TG neurons stimulated with 50 µg/mL f5, 300 µM ME, 220 mosmol/L HYPO, 50 µM MO, and 3.3 µM CAP. (**F**) Percentages of all viable TG neurons responsive to f5, ME, HYPO, MO, and CAP. As many TG neurons were multiply responsive, added percentages exceed 100%. (**G**) Diagram showing the coresponsiveness of all f5-sensitive TG neurons to one or several of the typical trigeminal stimuli ME, HYPO, MO, and CAP. More than half of all f5-sensitive TG neurons (brown inner circle) were also sensitive to HYPO (light orange area). Among those f5 and HYPO responsive neurons, about two-thirds were also sensitive to CAP (red area). Of the neurons sensitive to EGCG, HYPO, and CAP, about 20% were also activated by ME and MO (blue and yellow areas, respectively). (**H**) Effects of Ca^{2+} buffering or preincubation with different inhibitors on responses to f5. (**I**) Percentages of TG neurons responsive to 100 mg/mL f5 after preincubation with either DMSO or suramin. Sample sizes are given in parentheses. ***$P \leq 0.001$.

phenols like EGCG, typical of, for example, tea, would also sense polymeric tannins typical of red wine. For in vitro tests on TG neurons, polymeric tannins were isolated from a Bordeaux wine by means of ultrafiltration using a 5-kDa cutoff membrane as described recently (Wollmann and Hofmann 2013). The isolated polymers were separated by gel adsorption chromatography to give 8 subfractions (f1–f8). After thiolytic depolymerization, the mean degree of flavan-3-ol polymerization (mDP) and the percentage of galloylation in each fraction were determined by high-performance liquid chromatography with UV detection following literature protocols (Thompson et al. 1972; Preys et al. 2004). With the exception of f1, lacking any flavan-3-ols or galloyl moieties, a high degree of galloylation was found in f2–f8 (8.4–17.3% ECG units). In Ca^{2+} imaging experiments, f2–f8 and the complete HMM fraction at a concentration of 100 µg/mL elicited responses in 7.9% (f2) to 52.1% (f4) of TG neurons with f8 eliciting the largest mean responses (Figure 7A–C). Similar to the monomeric phenols, the polymeric wine astringents induced a sharp rise of intracellular Ca^{2+} levels directly after or within up to 10 s after stimulus onset that started to decline in the presence of the stimulus (Figure 7A,E). Among all subfractions tested, f4, f5, and the complete HMW fraction elicited reasonably large responses in robust fractions of TG neurons (Figure 7B,C). These responses did not sensitize or desensitize upon repetitive stimulation (Figure 7D). As the complete HMW fraction is a mixture of all other fractions and thus least homogeneous with respect to chemical structures, we chose f5 among f4 and f5 as the model fraction for further experiments. We consecutively stimulated TG neurons with 50 µg/mL f5 and the typical trigeminal stimuli CAP (3.3 µM), ME (300 µM), MO (50 mM), as well as with HYPO (220 mosmol) (Figure 7E). Of 394 TG neurons tested, 54.8% were sensitive to 50 µg/mL f5 (Figure 7F). Among these f5 responders, 10.4% did not respond to another stimulus, 18.6% to 300 µM ME, 55.8% to HYPO, 6.1% to 50 µM MO, and 67.1% to 3.3 µM CAP (Figure 7G), but none of the interactions was significant. The responses to f5 were not diminished in the presence of the unspecific TRP channel antagonist RR (10 µM) arguing against the activation of TRP channels by the wine polyphenol (Figure 7H).

In summary, polymeric red wine astringents containing galloyl groups stimulated responses in TG neurons. This finding suggests that monomeric as well as polymeric astringents with galloyl moieties can be detected by TG neurons. None of the TG subpopulations identified by typical trigeminal stimuli showed a higher probability of responsiveness to the red wine astringent f5.

A polymeric red wine astringent stimulates G protein–coupled signaling in TG neurons

We next investigated the signaling events induced by the red wine polyphenol f5 in TG neurons. When applied at an interstimulus interval of 2 min, 50 µg/mL f5 elicited repetitive responses of similar amplitude (Figure 7D). In Ca^{2+}-free buffer, the responses to f5 were almost completely blocked (Figure 7H). Similarly, responses were greatly reduced in the presence of a mixture of the VGCC blockers 10 µM nimodipine, 10 µM mibefradil, and 1 µM ω-conotoxin MVIIC (Figure 7H). After treatment with 100 µM suramin to dissociate the G protein to receptor coupling, 7% of the TG neurons responded to f5 (DMSO control: 46%, $P \leq 0.001$, Figure 7I). When coapplied with 100 µM of the adenylyl cyclase blocker SQ22536 or the CNG channel blocker L-*cis*-diltiazem (100 µM), responses to f5 were almost completely inhibited (Figure 7H). Coapplication of f5 with 500 µM of the CaCC blocker NFA reduced the mean response amplitudes by about 30% (Figure 7H).

Taken together, the responses of TG neurons to the red wine polyphenol f5 seem to be mediated by a G protein–coupled receptor, which signals down to adenylyl cyclase, CNG channels, and CaCCs.

Discussion

Astringency is a common sensory experience thought to be elicited by a 2-step mechanism involving the precipitation of salivary proline-rich proteins by phenolic compounds (Luck et al. 1994; Feldman et al. 1999; Edelmann and Lendl 2002; Jöbstl et al. 2004) and the stimulation of oral mechanosensors resulting from increased friction (Lyman and Green 1990; Luck et al. 1994; Feldman et al. 1999; Edelmann and Lendl 2002; Jöbstl et al. 2004). However, the question of whether the trigeminal or taste system mediates astringency perception remains a subject of debate. We evaluated the differential contributions of both sensory systems to astringency perception in psychophysical tests on human subjects with unilateral taste nerve lesion or after lidocaine anesthesia of 1) their taste nerve or 2) their taste and trigeminal nerves. In these experiments, astringency perception clearly was independent of taste nerve function. Only lidocaine anesthesia of both the CTN taste nerve together with the LN, the lingual branch of the trigeminal nerve (inferior alveolar nerve block), resulted in a near-complete loss of astringency perception on the tongue. Albeit using completely different approaches, the results of our psychophysical experiments support previous findings on human subjects that are in favor of trigeminal astringency perception (Breslin et al. 1993).

Several studies suggest that tannins negatively affect digestion and that specialized salivary proteins inactivate astringents by binding to them evolved as a countermeasure in herbivorous species (reviewed in McArthur et al. 1995; Shimada 2006; Barbehenn and Constabel 2011). Plants with high tannin content are avoided by different taxa, among them rodents (Shimada and Saitoh 2003) and primates (Takemoto 2003), and tannic acid was shown to elicit avoidance behavior in mice in a laboratory setting (Ramírez et al. 2011). The trigeminal sensory system initiates protective reflexes and avoidance behavior in response to harmful environmental stimuli. In accordance with the potential harmful effects of astringents, it appears plausible that our results classify astringency as a trigeminal percept in humans.

Astringency perception is facilitated but not completely dependent on oral movement (Breslin et al. 1993), which is supported by our observation that in the psychophysical study, subjects did not or only hardly perceive astringency without any tongue movement. However, the psychophysical functions of astringents do not necessarily reflect their capacity to precipitate proline-rich proteins (Hofmann et al. 2006). Moreover, some potent astringents do not precipitate salivary proteins at all (Schwarz and Hofmann 2008). Therefore, we tested whether astringents could directly activate TG neurons in vitro, which would indicate a chemosensory component of trigeminal astringency perception. We chose primary cultures of dissociated mouse TG for these experiments. As test substances, we used astringents isolated from food or beverages that were identified as the crucial determinants of the astringency of the respective natural products (Scharbert et al. 2004; Schwarz and Hofmann 2007; Hufnagel and Hofmann 2008a, 2008b). The concentrations of 250–1000 µM that we used in the in vitro experiments reflect those found in plant-derived foods, for example, black and green tea (Hara et al. 1995). Moreover, human detection thresholds for astringents range between 190 and 540 µM (Scharbert et al. 2004). Among the catechins we tested, several compounds robustly activated TG neurons. Interestingly, compounds with one or several galloyl moieties were much more potent activators than compounds lacking any galloyl moiety and most of the compounds without galloyl moiety did not activate TG neurons at all. In tendency, catechins with 2 or more galloyl moieties activated higher percentages of neurons than compounds with only 1 moiety. Albeit the exact structures of the astringent wine polymers we tested remain to be identified, composition analysis revealed a high mean degree of flavan-3-ol polymerization and a high percentage of galloylation in the subfractions that activated TG neurons in our experiments (Wollmann and Hofmann 2013). Importantly, the simplest galloylated astringent gallic acid acted as a robust stimulus of TG neurons, whereas protocatechuic acid that lacks only 1 hydroxyl group in comparison to gallic acid was not. Owing to these findings, we suggest the existence of specific galloyl receptors on TG neurons that are not or only weakly stimulated by chemicals without galloyl moieties. A rough and puckering astringency impression correlates with the degree of galloylation of a given astringent (Peleg et al. 1999; Chira et al. 2009; McRae et al. 2013). In concert with that, most galloylated astringents that activated TG neurons in our study elicit a prototypic rough and puckering astringency, whereas most nonactivating substances produce a soft, velvety astringent mouth coating (Scharbert et al. 2004; Hufnagel and Hofmann 2008b). Based on these data, it appears that subsets of TG neurons are selectively activated by highly puckering astringents that have at least 1 galloyl moiety. Nongalloyl astringents and velvety astringent molecules seem to be detected by yet elusive mechanisms. At this point, we can only speculate that the sensation of puckering astringency may be elicited by the coactivation of trigeminal chemosensors and mechanosensors, whereas velvety astringents might exclusively activate trigeminal mechanosensation. However, we used mouse neurons for the in vitro experiments and it is completely unknown whether rodents sense the different qualities of astringency. Simply put, mouse TG neurons seem to possess receptors for substances with galloyl moieties. These receptors are yet to be identified and may have correlates in human.

According to their sensitivity for specific TRP channel agonists, TG neurons can be grouped into different subpopulations. We tested which subpopulations of TG neurons are activated by EGCG and the red wine astringent f5. Most TG neurons sensitive for either EGCG or f5 were also sensitive for the TRPV1 agonist CAP and/or HYPO activating TRPV4, but only the interaction between EGCG and CAP sensitivity was significant. We suggest that not only TRPV1-positive but also TRPV1-negative neurons signal astringency to the brain. It is difficult to speculate at this point how the activation of different trigeminal fiber types will be processed centrally and finally result in the complex and unique sensation of astringency.

Despite the high overlap between responses to astringents and typical TRP channel agonists, we found no evidence for the direct activation of TRP channels by astringents, and more specifically, the activation of TRPA1 by EGCG that has been reported elsewhere (Kurogi et al. 2012). Thus, TRP channels appear not to be activated by the astringent plant compounds we tested. Instead, our results point to a G protein–coupled detection mechanism for astringent phenols initiated by a Gαs-coupled receptor with a high affinity for galloyl moieties. This receptor seems to signal down to adenylyl cyclase and CNG channels. As a possible channel in TG signaling, CNGA2 was lately identified in TG tissue by next-generation sequencing (Manteniotis et al. 2013). Further experiments and technical approaches should be applied to test our initial findings on the G protein–coupled signaling cascade.

In our experiments, about 22% of the TG neurons responded to 250 µM EGCG. Interestingly, about 25% of the responses to EGCG were not or only partially inhibited by the blockers suramin, SQ22536, and L-*cis*-diltiazem, which inhibit the functions of Gαs, AC, and CNG channels, respectively. This observation hints at an additional, G protein–independent detection mechanism for EGCG in at least a subpopulation of all EGCG-sensitive TG neurons. Previously, we could show Cl^--dependent signal amplification in TG neurons that depended on CaCCs after CAP application (Schöbel, Radtke, et al. 2012). In this study, responses to astringents were also sensitive to blockers of CaCCs. Together, these observations suggest that Cl^--dependent signal amplification is induced by several stimuli in TG neurons. According to our next-generation sequencing analysis of TG material, Ano8 and a non-Ano-type CaCC, namely Tweety 3, are most relevant for CaCC function in TG neurons (Schöbel, Radtke, et al. 2012).

In this study, we suggest the existence of a trigeminal G protein–coupled receptor for galloylated molecules. Which could be possible candidate receptors? Gallic acid and caffeic acid activate the G protein–coupled receptor GPR35 (Deng and Fang 2012), which couples to Gαi/o (Guo et al. 2008). However, Gαi/o-mediated events rather inhibit cellular activity and we did not find evidence for an involvement of Gαi/o. EGCG activates the 67-kDa laminin receptor at nanomolar concentrations (Tachibana et al. 2004). This receptor mediates cell adhesion and induces multiple intracellular signaling events (Nelson et al. 2008). Yet, 67-kDa laminin receptor signaling does not include the fast mobilization of Ca^{2+}. Bitter taste receptors are activated by several astringent compounds (Soares et al. 2013) and TG neurons are stimulated by highly concentrated bitter tastants (Liu and Simon 1998). However, in taste cells, bitter taste receptors signal down to Ca^{2+} release from the ER (Chaudhari and Roper 2010) that we did not observe in response to stimulation with EGCG. Furthermore, deep sequencing analysis did not reveal the presence of bitter taste receptors in TG neurons (Manteniotis et al. 2013, database research). The relatively newly discovered mas-related G protein–coupled receptors (Mrgprs) are involved in the perception of pain, itch, and massage-like stroking (Liu et al. 2009; Rau et al. 2009; Wilson et al. 2011; Vrontou et al. 2013). Several Mrgprs are highly expressed in TG tissue (Manteniotis et al. 2013), but they are yet to be deorphanized. Due to their high abundance in TG neurons and G-protein coupling, they represent candidate receptors for galloyl-type astringents. However, we think it is unlikely that astringents might induce the same downstream activation of a TRP channel upon Mrgpr activation that was described previously (Wilson et al. 2011) as the responses to astringents were insensitive to the TRP channel blocker ruthenium red in our experiments. The possible interaction of astringents with Mrgprs clearly requires further research.

In summary, our study shows that astringency is a trigeminal percept in human subjects. Furthermore, we describe the activation of TG neurons by astringent phenols with one or several galloyl moieties. First evidence points to a common signaling cascade that is activated by 2 structurally diverse astringents in these cells. These observations indicate a specialized chemosensory detection mechanism for astringents in TG neurons. We are aware of the fact that in our psychophysical experiments, astringency perception was greatly facilitated by oral movement. We can only speculate that the hydrophilic astringent phenols that stimulate responses in trigeminal sensory neurons in vitro might require mechanical costimulation in vivo, at least in humans. Possibly, upon consumption of polyphenol-rich alimentary products, the chemosensory detection of astringent phenols together with the stimulation of trigeminal mechanosensors creates the entire sensation of astringency. Future studies should address this possible synergism between a chemosensory and mechanosensory activation of trigeminal sensors by astringents. Beyond that, studies should address the identity of a presumed trigeminal galloyl receptor.

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Conflict of interest

J.P.L. is employed by Symrise AG. The other authors declare that there are no other conflicts of interest.

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