Supporting Information

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SI Methods

Subjects. ***Experiments in awake and behaving animals.*** Thirty-seven male Long–Evans rats were obtained from Charles Rivers Laboratories and housed individually in Plexiglas cages. Seventy-nine C57BL/6 mice homozygous for a partial deletion of the *Trpm5* gene (KO) (1), were bred from animals generously donated by C. S. Zuker (UCSD, San Diego, CA). Eighty C57BL/6 wild-type (WT) mice were obtained from the Jackson Laboratory. Both male and female mice were used for data collection and sex distribution was balanced in each experiment. Genotype was confirmed by PCR amplification of the *Trpm5* gene (1). Mice were housed in Plexiglas cages in groups of 2 to 5. All animals were maintained on a 12 h light/dark schedule and experiments were carried out in the light portion of the cycle. At the time of experiments, animals were 3 to 6 months old and naïve for the tastants used in that experiment. Purina rodent chow and water was available ad libitum, except for the duration of behavioral testing, when animals were water deprived. All procedures were approved by the Duke University Institutional Animal Care and Use Committee.

Experiments in anesthetized animals (Chorda Tympani recordings). Nine female Sprague–Dawley rats and 12 female C57BL/6 mice (6 KO and 6 WT) were used. All procedures were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Stimuli. All tastant solutions [monosodium glutamate (MSG), 75, 100 and 300 mM; NaCl, 75, 100 and 300 mM; nicotine free base, 0.03, 0.1, 0.3, 0.5, 1, 3, 5, 10 and 20 mM; NH_4Cl, 300 mM; quinine HCl, 0.01, 0.03, 0.1, 0.2, 0.3, 1, 3 and 10 mM; sucrose, 75, 100 and 300 mM; SC45647, 5 mM] were prepared daily in distilled water. Solutions were maintained at room temperature and pH ranged from 5 to 8. Distilled water was also used as baseline stimulus (so that every mention of "water" in the article implies that distilled water was used). In some cases, mecamylamine, a broad spectrum nicotinic acetylcholine receptor (nAChR) antagonist (2), was dissolved with tastant solutions or water to obtain final concentrations of 0.05, 0.1, 0.2, 0.3, 0.4 or 0.5 mM. In one experiment, 1% DMSO was prepared in distilled water and used both as a base stimulus and also to solubilize capsaicin at 0.01 mM. All chemicals were obtained from Sigma–Aldrich and were reagent-grade.

Behavioral Measurements. All behavioral tests were conducted in Med Associates behavior boxes, each enclosed in a ventilated and sound-attenuating chamber. Chambers were equipped with one or two slots for sipper tubes in one of the walls. Access to sipper tubes could be blocked by computer-controlled doors. Some of the sipper slots were equipped with licking detection devices with 10 ms resolution (Med Associates). Either contact or beam lickometers (necessary in animals where neural activity was recorded during behavior) were used for licking detection (Med Associates). Mouse beam lickometers were custom-made (3) and used only for brief access tests. Rat cages were also equipped with 2 levers for instrumental tasks. With a single exception noted below, before testing, animals were water deprived for ≈22 h. After behavioral testing, they were given 1 h of free access to water in their home cage. Before the start of each test, animals were habituated to the deprivation schedule and the behavioral chamber that was used in each case. In these habituation sessions animals were given access to sippers but only water was available.

Mouse 2-bottle preference tests. Mice were tested in 30 min long 2-bottle choice tests. Animals were presented with 2 bottles to which they had constant free access during the duration of each experiment. The number of licks for each sipper was recorded and used to calculate preference ratio (see *SI Appendix*). To reduce confounds produced by potential side-biases or posting-estive effects (4), mice were tested in each condition for 4 consecutive days with daily inversion of bottle positions for each tastant. Preference for 10 mM quinine vs. water (11 KO and 9 WT), 0.5 mM nicotine vs. water (9 KO and 10 WT), 0.5 mM nicotine vs. 10 mM quinine (9 KO and 10 WT), 1 mM nicotine vs. water (9 KO and 10 WT) and 1 mM nicotine vs. 10 mM quinine (9 KO and 9 WT) was tested. In the animals tested with 0.5 mM nicotine vs. water, 4 days of additional testing with a higher concentration of nicotine (1 mM) vs. water were conducted after the first 4 days of preference testing.

Two-bottle preference tests in capsaicin-treated Trpm5$^{-/-}$ mice. To investigate the contribution of somatosensory input to the preference for nicotine, 14 KO mice were injected with capsaicin in the neonatal period, following previously described methods (5). The mice were injected s.c. with 50 mg/kg capsaicin (in 10% ethanol–10% Tween 80) at the second or third postnatal day and tested for nicotine preference as adults. Two bottle preference tests were run consecutively for 8 days, with preference for 0.5 mM nicotine vs. water tested in the 1st 4 days and for 1 mM nicotine vs. water in the last 4 days. Because the effectiveness of neonatal capsaicin treatment may vary (6), an additional experiment was performed to exclude animals with normal measures of orosensory reactivity to capsaicin, i.e., those where the effectiveness the neonatal treatment was presumably suboptimal. Thus, once nicotine preference was established, 2 additional days of testing were performed to verify preference for 0.01 mM capsaicin. Capsaicin was dissolved in 1% DMSO and tested vs. 1% DMSO. Capsaicin preference in treated KO mice was then compared with that of 10 untreated KO mice with similar prior experience with nicotine. Data from 5 treated KO mice was excluded because capsaicin preference in these animals was lower than the average capsaicin preference in untreated animals.

Mouse brief access tests. Mice were tested in 30 min long brief access tests, conducted as described previously (4, 7). Briefly, each animal had access to only one sipper to which it was given intermittent access in sequential trials. After the animal's first lick in any trial, the sipper would deliver 1 aliquot (≈3 μL) of a tastant for each detected lick response. Five seconds after the first lick, access to the sipper was blocked for an intertrial period of 7 seconds, after which a new trial with a different tastant was initiated. A computer-controlled and gravity driven valve system (ALA Scientific) randomly presented 4 different test stimuli within blocks of 4 trials, 1 tastant per trial. The cumulative number of licks for all trials of each tastant was recorded and used to calculate the respective lick ratio (see *SI Appendix*). 9 KO and 11 WT animals were tested with water (reference stimulus), 10 mM quinine and 2 nicotine solutions (0.5 mM and 1 mM). Another group of 9 WT mice was tested with water (reference stimulus), 0.3 mM mecamylamine, 10 mM quinine and a mixture of 10 mM quinine with 0.3 mM mecamylamine. To reduce variability, in these groups, each animal was tested several days to obtain average lick ratios for each tastant. 9 KO and 13 WT naïve animals were tested with water (reference stimulus) and 3 nicotine solutions (0.5, 1 and 3 mM). On alternate days, the same

solutions were used but 0.3 mM mecamylamine was mixed with water and all stimuli.

Rat 2-bottle preference tests. Twenty male rats were tested in 2 behavior boxes, each with 2 sipper slots for presentation of taste solutions in graduated 50-mL cylinders. Ten rats were used to test preference for nicotine vs. water and the remaining the preference for quinine vs. water. Each rat was tested in daily 15 min long sessions where one of the sippers allowed consumption of a particular stimulus (i.e., a concentration of one of the tastants - nicotine or quinine) and the other sipper contained water. After each session, the volume of the stimulus solution and of water that had been consumed were measured in a graduated cylinder and used to calculate the preference ratio (see *SI Appendix*). The method of ascending limits was used to present several tastant concentrations (8). Concentrations of quinine HCl ranged from 0.01–3 mM and nicotine concentrations ranged from 0.03–10 mM, both presented in ascending half-log steps. Each stimulus was tested in 2 sessions such that the position of the tubes containing the tastants could be alternated to account for side bias. After a particular concentration had been tested twice, the next concentration was tested until the highest concentrations had been tested twice. A washout day, where water was presented in both tubes, was conducted between each session where a stimulus was presented.

Rat 2-alternative choice tests. Nine male rats were trained and tested in a tastant discrimination test, with a similar protocol to what has been previously described (9, 10). Behavioral chambers were equipped with 2 sipper tubes and 2 levers. Access to sipper tubes was controlled by computer-controlled doors. Each sipper tube contained a bundle of several 20-gauge stainless-steel tubes, cemented together such that there was no dead space in which tastants might mix. All taste solutions and distilled water were contained within 50-ml chromatography columns (Kontes Flex-Columns; Fisher Scientific), and the system was maintained under ≈8 psi of air. Computer-controlled solenoids (Parker Hannifin Corporation, Fairfield, NJ) regulated the flow of fluid from the columns through tubing connected to the sipper tube. The columns and solenoids were housed outside the sound-attenuating chambers. Within 10 ms after a lick was detected (due to breaking of an infrared beam), 1 of the valves opened and delivered 25 to 50 μL of fluid. Rats were trained in this setup to differentially press one of the 2 levers depending on whether the stimulus delivered in the stimulus sipper was nicotine or quinine, delivered in a single lick after 4 dry licks (FR5 schedule). If the rat responded correctly, it received immediate access to a reward sipper to receive water reinforcement (maximum 10 licks or 5 seconds reinforcement period). An incorrect response resulted in a time-out that served as a punishment. After receiving the probe tastant, the rats had a limited hold period that was signaled by cue lamps above the levers. If the limited hold period expired with no response, the rat received a time-out. Training consisted of 5 phases: alternation, random, discrimination training 1 (DT1), DT2, and DT3. The first two phases were conducted with only one intermediate concentration of each tastant (1 mM nicotine and 0.2 mM quinine). During the alternation phase, a criterion number of correct lever presses to a tastant was required before the other tastant was presented, and vice versa. This criterion number was systematically reduced across sessions. During the random phase, stimuli were randomly presented. During DT1, DT2, and DT3, session parameters were gradually changed to a shorter limited hold period (down to 5 seconds) and longer time-out periods (up to 30 seconds). Importantly, the number of stimulus concentrations was increased from 1 to 3 for each tastant to render intensity an irrelevant cue. The 3 concentrations of each tastant were presented in randomized blocks of 6 (without substitution). The animal could initiate as many trials as possible in each 60 min session. Rats were maintained on 23 h restricted water access schedule for 5 consecutive days a week and free water access was given in the home cage for the remaining 2 days. Any animal whose body weight dropped to <90% of the value during free water access was given supplemental water. Two rats were unable to learn this task and were dropped from testing. In the remaining animals, once the percentage of correct responses were above chance for at least 5 consecutive days, they were tested with water in all valves to check if the orosensory properties of the tastants were the sensory characteristic used for discrimination.

Rat 2-alternative choice tests—effect of mecamylamine. The 7 rats that were successfully trained to discriminate between nicotine and quinine were, after a period of rest, retrained on the task. They were then tested on a session when nicotine solutions were made with the use of 0.3 mM mecamylamine as the solvent. This test session was conducted in counterbalanced order with a control session where all nicotine solutions were replaced by water, to test whether rats treated mecamylamine-adulterated nicotine as if it were water. In the test and control sessions, to avoid any intrasession learning, the access to water was not contingent upon the animals' response but, rather, was given with a fixed 0.8 probability after every lever press.

Chorda Tympani (CT) Taste Nerve Recordings. CT nerve recordings were performed following previously described protocol (11, 12). Briefly, animals were anesthetized with pentobarbital (60 mg/kg for induction and 20 mg/kg supplements as necessary) and body temperature was maintained at 37 °C with a thermal blanket. The left CT nerve was exposed laterally as it exited the tympanic bulla and placed onto a 32G platinum/iridum wire electrode to detect whole nerve responses to stimulation of the lingual surface. The anterior lingual surface was stimulated with rinse (water), control stimuli (300 mM NaCl; 300 mM NH_4Cl) and test stimuli, each of which remained on the tongue for 1 to 2 min. To assess preparation stability, control stimuli were applied at the beginning and end of the experiment. Rinse was applied between every stimulus application. In 3 rats and 6 mice (3 KO and 3 WT) test stimuli were 1, 3, 5, 10 and 20 mM nicotine, applied in a series of increasing concentration dissolved in water. This series was then repeated with the same stimuli dissolved with 0.3 mM mecamylamine. In 3 other rats, the test stimulus was 10 mM nicotine that was applied in the presence or absence of mecamylamine (0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mM). In this case, to normalize the contribution of mechanical flow to the phasic component of the response, solutions were flowed into a chamber affixed to the rat tongue at a rate of 1 mL/sec and each stimulus was applied several times to obtain a mean response in each subject (13). Three additional rats were tested with 10 mM nicotine, 100 mM NaCl and 5 mM SC45647 (a potent artificial sweetener) in the presence or absence of 0.3 mM mecamylamine. Also, to verify the stability of CT responses after use of mecamylamine, application of 10 mM nicotine was repeated at the end of the experiment. Finally, in another control experiment with 3 KO and 3 WT mice, 10 mM quinine was used as test stimulus. In WT mice, 10 mM quinine was applied in the presence or absence of 0.3 mM mecamylamine. Responses were integrated as described previously and digitized and analyzed offline (13). Once the experiment had finished, animals were killed with i.p. injection of 100 mg/kg pentobarbital.

Reverse transcriptase PCR for nAChR Subunits. Fungiform and circumvallate taste buds were harvested from isolated rat lingual epithelium and individually collected using a patch pipette of ≈100 μm pore according to previously described methods (14). The CT nerve was surgically exposed in its exit from the tympanic bulla and a small fragment was collected. Total RNA was extracted using an RNeasy kit (Qiagen) and the cDNA was generated and amplified using M-MLV Reverse Transcriptase

(Invitrogen) in a PTC200 DNA Engine (MJ Research). PCR screening of the cDNA for the presence of α-3, α-4, β-2, and β-4 nAChR subunits was done using already described rat primers (15) supplied by IDT. Positive controls were performed for alpha-gustducin and beta-actin. Negative controls were milliQ water.

Gustatory Cortex Neuronal Recordings. Gustatory cortex (GC) neuronal recordings were conducted according to previously described protocol (16). Procedures are described briefly.

Electrode implantation. Eight naïve rats were implanted bilaterally with moveable electrode bundles (16 15 μm tungsten microwires per cannula shaft) above GC (1.3 mm anterior, 5.2 mm lateral, and 4.7 mm horizontal from Bregma). After 1 week of recovery, the electrodes were lowered 250 μm per day until reaching the GC.

Neuronal recordings. Simultaneous recording of neuronal and licking activity commenced once the GC had been reached. Differential recordings were fed into a parallel processor that simultaneously digitized the analog signals from all 32 channels at 40 kHz (Plexon Inc., Dallas, TX). Discriminable action potentials with a signal/noise ratio $\geq$3:1 were isolated on-line from each channel by means of voltage-time threshold windows and a 3 principal component contour template algorithm. The refractory period for single units was set at 1.5 ms. Time-stamped records of stimulus onsets, spiking events, and all spike waveforms were stored digitally for additional offline sorting. In 4 animals, the electrodes were lowered further in GC and a second session was recorded on another day, such that a total of 12 sessions were obtained. Throughout each experiment, neural activity was recorded continuously.

FR5 schedule. As described in ref. 16, rats were allowed to lick to obtain water or a stimulus solution under a fixed ratio schedule (FR5). The setup used was the one described above for the 2-alternative choice test. Briefly, the animals were trained to lick a dry sipper spout where 50 μL of a given stimulus were delivered every 5th detected lick. Dry licks served as controls for somatosensory responses. Each stimulus was delivered in blocks of 8 deliveries (i.e., 40 licks), and the order of stimulus blocks was randomized without replacement using a Latin Square protocol. Between blocks, 1 or 2 water "washouts" (50–100 μL) were delivered with a minimum interval of 5–10s from both the previous and subsequent block. Within a given testing session subjects were presented with 5 different tastants, in most cases at several concentrations, and water, such that 10 to 12 different stimuli were presented in multiple trials (3–8) for a total of 24–64 deliveries of each. These stimuli included sucrose (75, 100 and 300 mM), MSG (75, 100 and 300 mM), NaCl (75, 100 and 300 mM), quinine (0.1, 0.2 and 0.3 mM), nicotine (0.3, 1, and 3 mM) and water. In each session, 1 or 2 concentrations of both nicotine and quinine were tested and the remaining stimuli served mainly to increase the animals' motivation to complete multiple trials. The concentrations for nicotine and quinine were approximately matched for intensity from the results in the 2-bottle preference tests. In addition to being used as a rinse, water was also considered to be a tastant (17). In 2 sessions, after 2 blocks of deliveries for all stimuli had been completed, water was replaced by a mecamylamine solution and nicotine solutions were replaced by solutions of nicotine plus mecamylamine. The subjects were then retested on the FR5 schedule. The mecamylamine concentration (0.3 mM) was 10% of the highest nicotine concentration used and chosen in accordance to results in CT nerve recordings.

Histology. After completion of the experiments, rats were deeply anesthetized and perfused transcardially with formalin (10%). Brains were removed, tissue was sectioned through GC in 50-μm coronal slices and sections were stained with cresyl violet to visualize cell bodies and electrode tracks, allowing for verification of correct electrode placement (16). Electrodes were correctly implanted into the GC in all cases.

See *SI Appendix* for details on data analysis.

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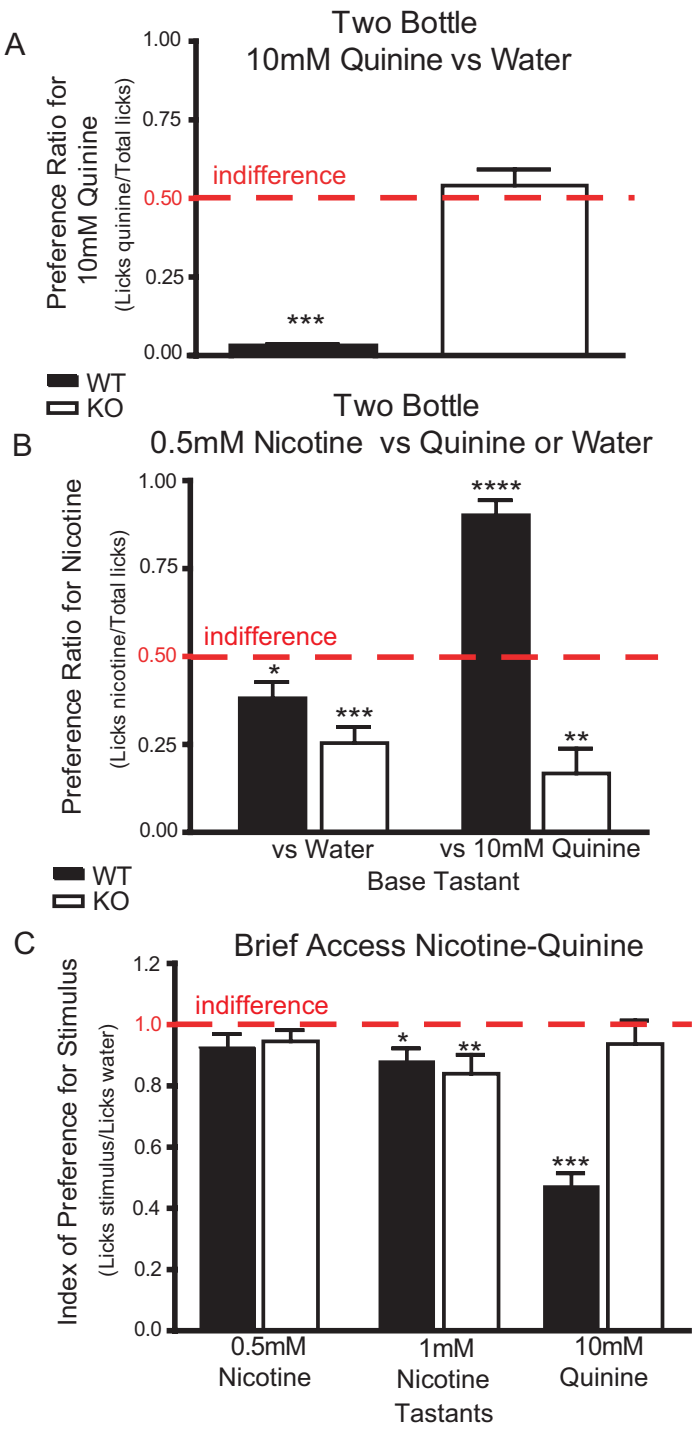

Fig. S1. Two-bottle and brief-access preference tests in mice. (*A*) Preference for 10 mM quinine (mean ± SEM) was measured against water in 2 bottle tests (11 KO and 9 WT mice). Although quinine was highly aversive (i.e., preference ratio significantly <0.5) for WT, it was indifferent for KO (***, $P < 0.0001$ and $P > 0.4$ respectively, independent 1-sample *t* tests vs. 0.5, Bonferroni–Holm's; red dashed line at the 0.5 indifference ratio). Accordingly, preferences differed among genotypes ($P < 0.0001$, unpaired 2-sample *t* test). (*B*) Two-bottle tests were used to measure preference (mean ± SEM) for 0.5 mM nicotine vs. water (9 KO and 10 WT) and vs. 10 mM quinine (9 KO and 10 WT) in naïve animals. With the exception of nicotine being preferred to quinine in WT mice, in all cases nicotine was aversive (*, $P < 0.04$; **, $P < 0.002$; ***, $P < 0.0008$, ****, $P < 0.0001$, independent 1-sample *t* tests vs. 0.5, Bonferroni–Holm's; red dashed line at the 0.5 indifference index). Thus, genotype, reference tastant and their interaction were found to have effects on preference ($P < 0.0001$, $P < 0.0009$ and $P < 0.0001$ respectively; 2-way ANOVA), with differences between genotypes only when nicotine was tested against quinine ($P < 0.001$, Bonferroni). Given the interaction between main effects, data were analyzed separately for each genotype showing differences in preference for nicotine tested vs. water or vs. quinine in WT ($P < 0.0001$) but not in KO animals ($P > 0.3$). (*C*) Brief access tests were used to confirm taste-dependent preferences for nicotine (0.5 and 1 mM) and quinine (10 mM) in 9 KO and 9 WT mice. Lick ratios (number of licks for stimulus normalized to the number of licks for water) were used as an index of preference for each stimulus (ordinate; expressed as mean ± SEM). 1 mM nicotine was aversive (i.e., lick ratio significantly <1) whereas 0.5 mM nicotine was indifferent for both genotypes. 10 mM quinine was aversive only for WT (*, $P < 0.0125$; **, $P < 0.002$; ***, $P < 0.0001$; 1 sample *t* tests vs. 1, Bonferroni–Holm's; red dashed line at the 1 indifference index). Thus, significant main effects on index of preference were found for genotype, stimulus and their interaction ($P < 0.002$, $P < 0.0001$ and $P < 0.0001$; 2-way, repeated measures ANOVA), with differences between genotypes only for quinine ($P < 0.001$, Bonferroni). We thus confirmed that both KO and WT mice avoid the taste of nicotine whereas only WT animals find quinine aversive.

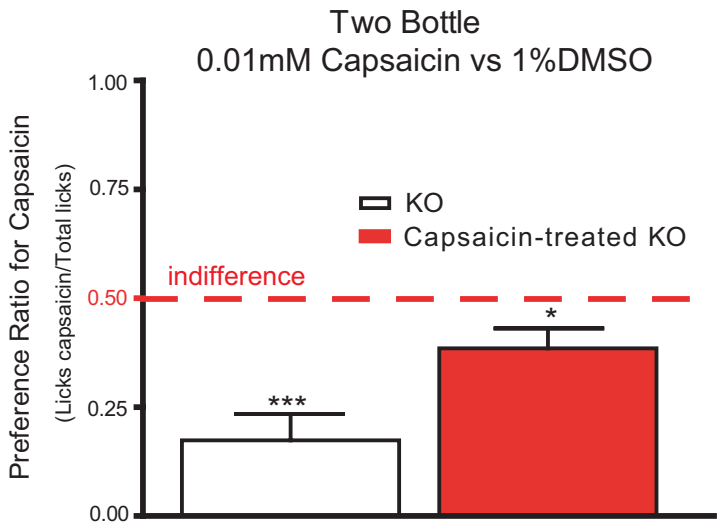

Fig. S2. Efficacy of neonatal capsacin treatment. Preference for 0.01 mM capsaicin (mean ± SEM) was tested vs. vehicle (1% DMSO in distilled water) in 2-bottle tests. Capsaicin was aversive for both untreated KO mice and KO mice that had been injected with capsaicin neonatally (*, $P < 0.04$; ***, $P < 0.0005$, independent 1-sample *t* tests vs. 0.5, Bonferroni–Holm's; 10 and 9 animals respectively; red dashed line at the 0.5 indifference index). Preference was significantly different between the 2 groups ($P < 0.02$, unpaired 2-sample *t* test), demonstrating that capsaicin treatment was effective in eliminating capsaicin-sensitive fibers.

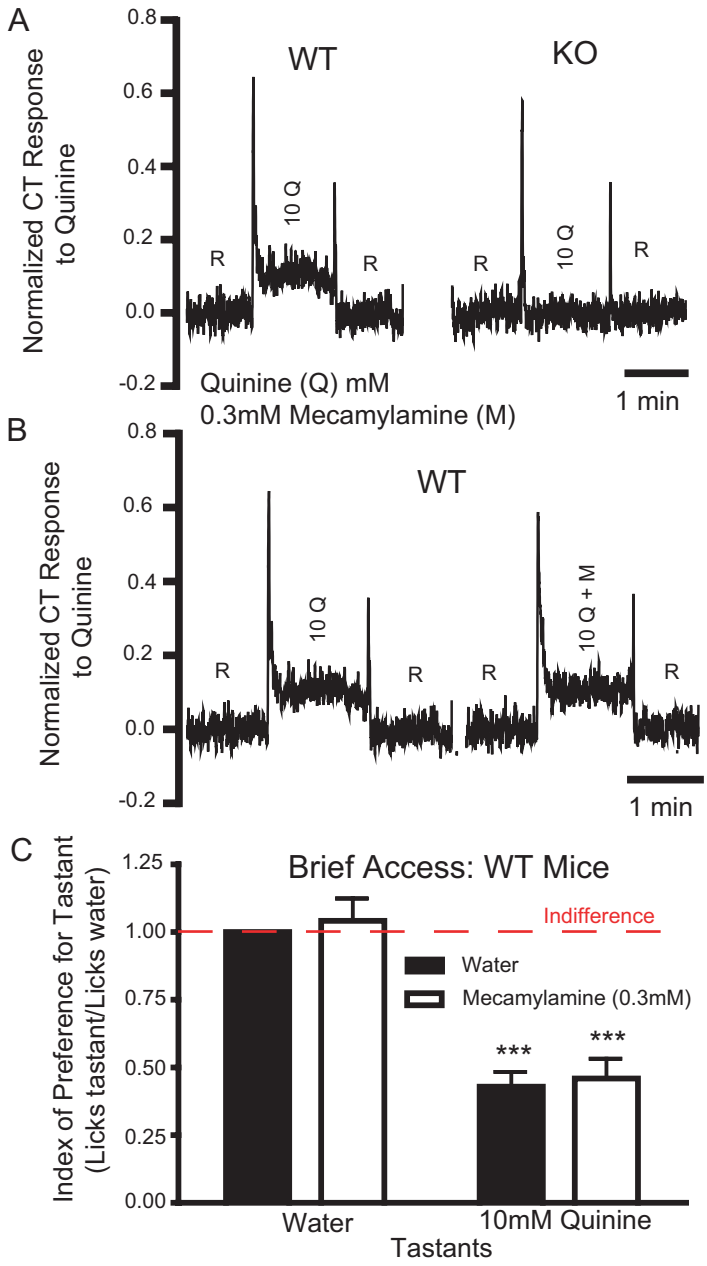

Fig. S3. Effects of mecamylamine on CT and behavioral responses to quinine. (*A*) Representative CT recordings evoked from a WT (left trace) and a KO (right trace) mouse in response to 10 mM quinine (see also Fig. 3*C*). (*B*) Example CT response to 10 mM quinine obtained from a WT mouse. Mecamylamine had no effect on responses to quinine (see also Fig. 4*C*). (*C*) WT mice ($n = 9$) were used in brief access tests to verify behavioral effect of nAChR antagonism in responses to water and 10 mM quinine. Four stimuli were presented in a single session: water, 0.3 mM mecamylamine, 10 mM quinine and 10 mM quinine + 0.3 mM mecamylamine. Lick ratios (number of licks for stimulus normalized to the number of licks for water) were used as an index of preference for each stimulus (expressed as mean ± SEM), such that index of preference for water was always 1. Only quinine solutions were aversive (***, $P < 0.0001$, 1 sample *t* tests vs. 1, Bonferroni–Holm's; red dashed line at the 1 indifference index; see lick ratios and statistical details in Table S1). Significant main effects on index of preference were found for stimulus ($P < 0.0001$) but not for mecamylamine or their interaction ($P > 0.4$ and $P > 0.9$ respectively; 2-way, repeated measures ANOVA). Thus, mecamylamine had no effects in behavioral responses to water or quinine in WT mice.

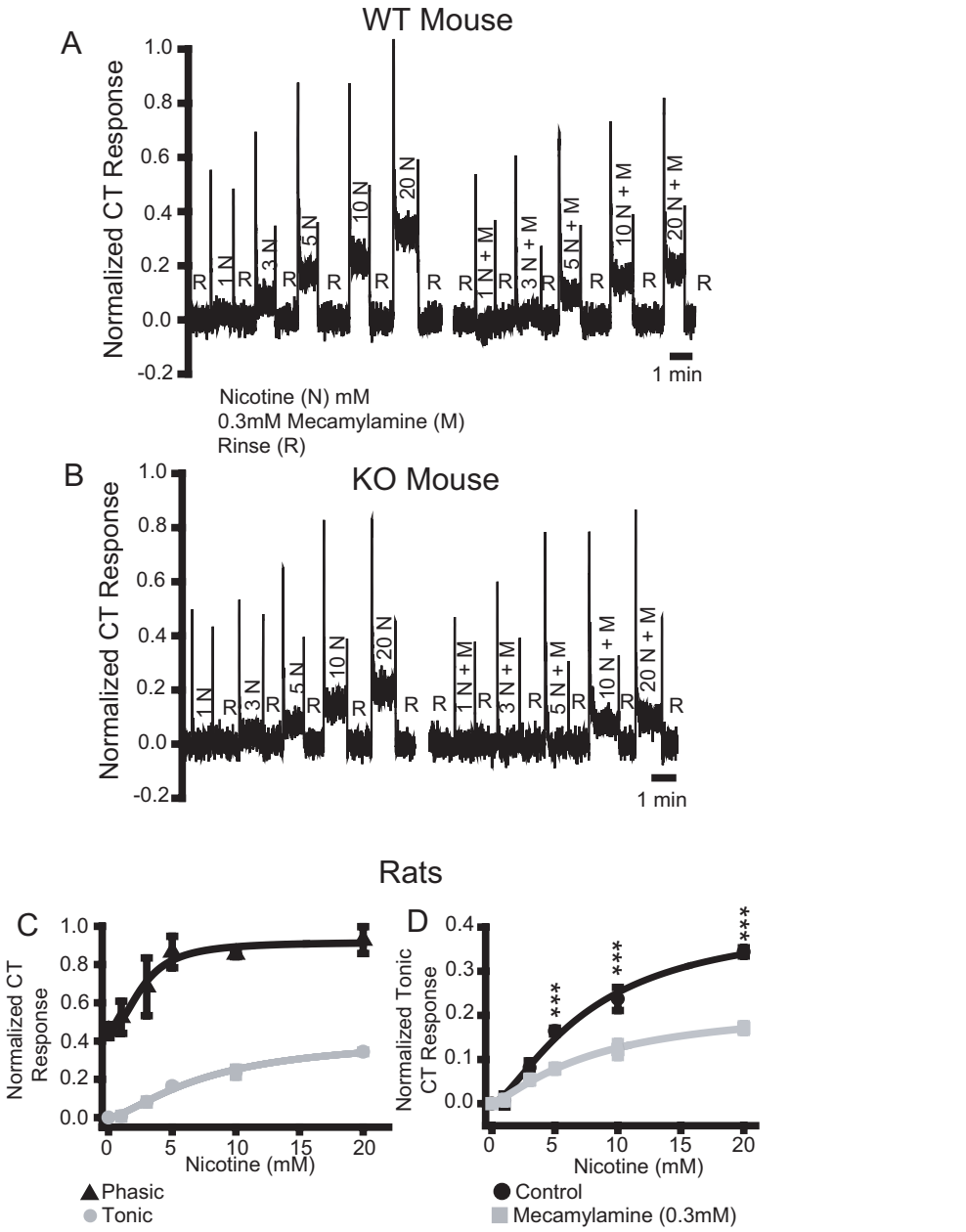

Fig. S4. Effects of mecamylamine on CT responses to nicotine. (*A*) Example CT responses obtained from a WT mouse with ascending concentration nicotine (*N*) stimulation series (1, 3, 5, 10 and 20 mM) in the presence or absence of 0.3 mM mecamylamine (*M*). Mecamylamine reduced phasic and tonic responses to nicotine (see Fig. 3*A*). (*B*) Same as in *A* but in a *Trpm5*-KO mouse. Again, 0.3 mM mecamylamine reduced responses to nicotine (see Fig. 3*B*). (*C*) Dose-response curves for both the phasic (black curve; $n = 2.19$, $EC_{50} = 2.74$ mM, $R^2 = 0.94$) and tonic (gray curve; $n = 1.46$, $EC_{50} = 7.68$ mM, $R^2 = 0.98$) components of the responses to nicotine were obtained in 3 rats (mean normalized CT response ± SD). Note that there is phasic response to 0 mM nicotine (i.e., rinse, which was water). (*D*) Mean tonic CT response (± SD) in the same rats as in *C* is shown for stimulation with nicotine alone (black curve, see above) and nicotine with mecamylamine (gray curve, $n = 1.26$, $EC_{50} = 8.21$ mM, $R^2 = 0.99$). Responses were significantly inhibited by mecamylamine ($P < 0.0001$ for mecamylamine, nicotine concentration and interaction; 2-way repeated measures ANOVA), particularly >3 mM nicotine (***, $P < 0.001$; Bonferroni).

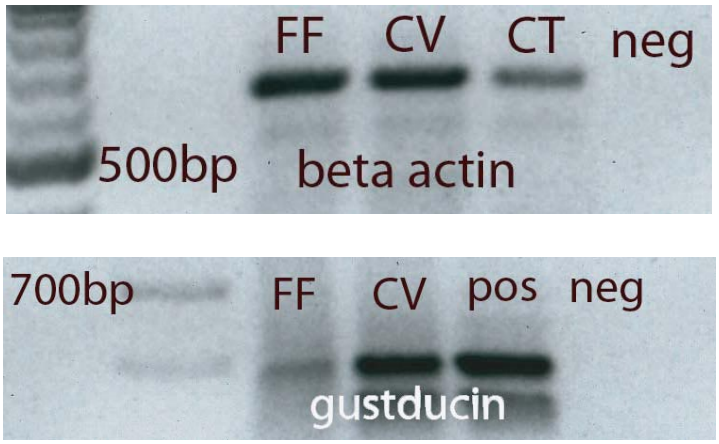

Fig. S5. Positive controls for RT-PCR were performed for beta-actin and alpha-gustducin in fungiform taste buds (FF), circumvallate taste buds (CV) and chorda tympani nerve (CT–also see Fig. 4*E*). Gustducin was also tested in an additional sample from isolated circumvallate taste buds (pos). Beta-actin was found in all tissues whereas expression of alpha-gustducin was only found in taste buds. Negative controls (neg) were miliQ water.

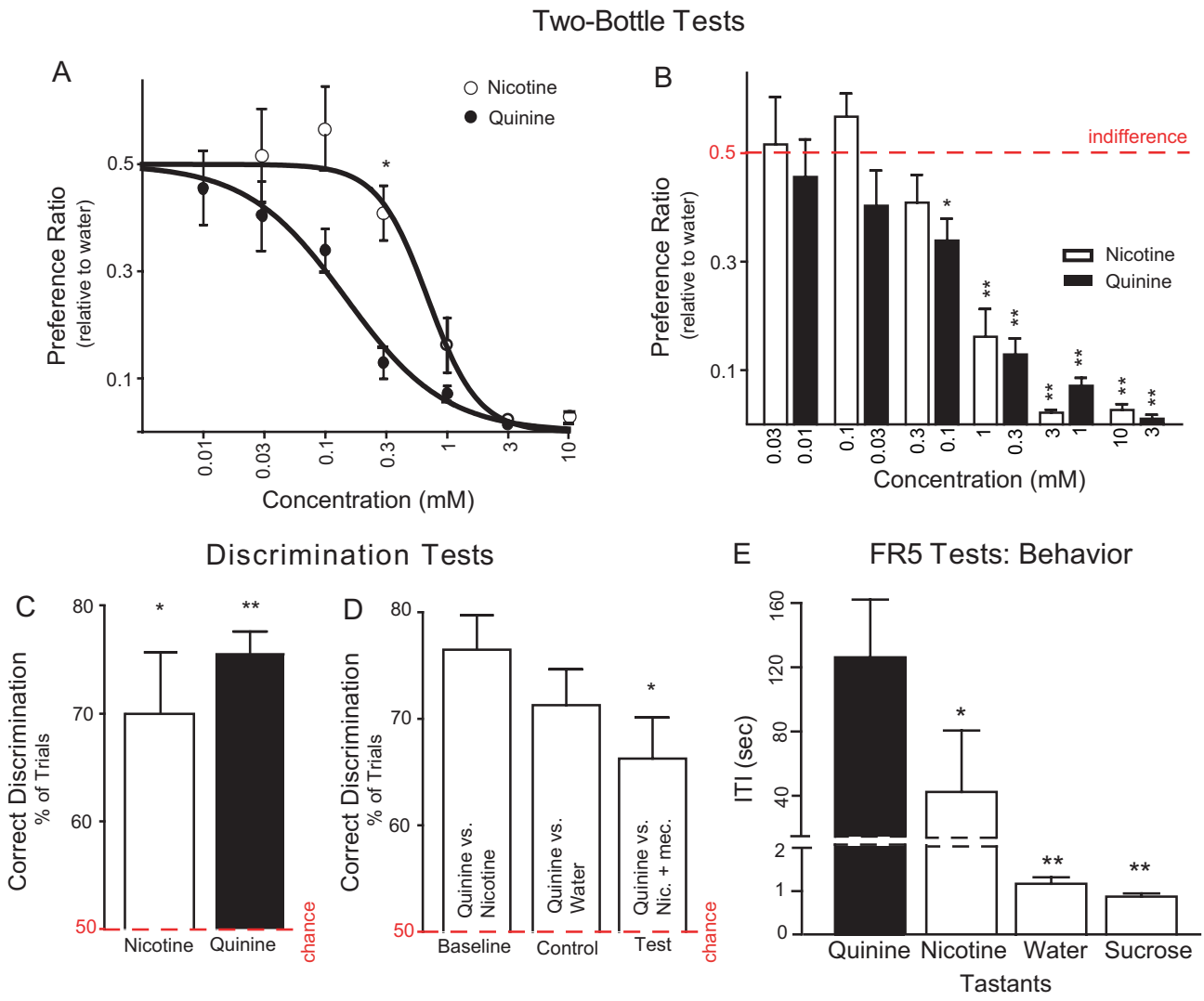

Fig. S6. Behavioral discrimination between nicotine and quinine in rats. (*A*) Preference for nicotine and quinine was measured in 2 separate groups of 10 rats using 2-bottle tests with ascending tastant concentrations vs. water. Preference ratios were higher for nicotine than quinine and decreased in a concentration dependent manner (tastant - $P < 0.002$, concentration - $P < 0.0001$, interaction - $P > 0.05$; 2-way, repeated measures ANOVA). A significant difference between tastants was found at 0.3 mM (*, $P < 0.05$, Bonferroni). To characterize concentration-rejection profiles for each tastant, preference ratios were fitted to sigmoidal functions (black curves; nicotine: n = -2.1, $R^2 = 0.62$; quinine: n = -1.1, $R^2 = 0.61$). Half-maximal rejection (EC_{50}) was at 0.15 mM for quinine and 0.69 mM for nicotine. (*B*) When data were reanalyzed with a half-log adjustment of concentrations (higher nicotine matched to lower quinine), preferences did not differ (significant main effect for concentration, $P < 0.0001$, and non-significant effects for both tastant, $P > 0.15$, and interaction, $P > 0.35$). Nicotine was aversive at 1, 3 and 10 mM whereas quinine was aversive at 0.1, 0.3, 1 and 3 mM (*, $P < 0.0032$ and **, $P < 0.0001$; independent 1-sample *t* tests vs. 0.5; red line at the 0.5 indifference ratio). From these data, several concentrations of each tastant (0.1, 0.2 and 0.3 mM for quinine and 0.3, 1 and 3 mM for nicotine) were chosen for further studies to allow comparisons of responses to these tastants at similar levels of behavioral aversion. (*C*) Discrimination between nicotine and quinine was tested in 7 rats with isointense nicotine and quinine concentrations (see main text). The percentage of correct responses was 70 ± 5.7 and 75.5 ± 2.1 for nicotine and quinine trials respectively, and was, in both cases, significantly above chance level (50% - red dashed line; *, $P < 0.02$; **, $P < 0.0001$; 1 sample *t* tests vs. 50%, Bonferroni–Holm's). (*D*) The 7 rats were retested in a baseline and then in control and test sessions, where nicotine was replaced respectively by water or by mixtures of nicotine and 0.3 mM mecamylamine. Overall correct discrimination differed significantly across these sessions ($P < 0.015$; 1-way repeated measures ANOVA). In fact, when compared with baseline, discrimination was significantly lower in the test session (76.5 ± 3.2% and 66.3 ± 3.9% respectively; *, $P < 0.05$) but not in the control session (71.9 ± 3.4%; $P > 0.05$; Newman-Keuls). (*E*) Neural and behavioral responses to multiple tastants were obtained simultaneously while these were delivered on a fixed ratio of 5 licks (i.e., tastant delivered at each lick after 4 consecutive dry licks). Intertastant intervals (ITI, measured in seconds) were used to quantify reactivity for each tastant. The mean lick rate in rats is 6–7 Hz [Gutierrez R, Carmena JM, Nicolelis MA, Simon SA (2006) Orbitofrontal ensemble activity monitors licking and distinguishes among natural rewards. *J Neurophysiol* 95(1):119–133] such that, with this experimental design, the lower theoretical ITI is ≈0.75 sec. ITI's (mean ± SEM) for water (1.17 ± 0.15), sucrose (0.88 ± 0.07), nicotine (42.37 ± 38.25) and quinine (126.2 ± 36.04) were different overall ($P < 0.004$; 1-way, repeated measures ANOVA) with pairwise differences only between quinine and all other tastants (*, $P < 0.05$ and **, $P < 0.001$, Newman-Keuls). Average concentration of presentation for nicotine and quinine (weighted according to number of times each concentration was presented–see *SI Methods*) was 1.14 mM and 0.23 mM respectively, and thus comparable to EC_{50} values obtained in 2-bottle tests (see *A*; 1.14/0.23 = 4.96 and 0.69/0.15 = 4.6). However, behavioral reactivity to the 2 tastants differed significantly, with intertastant intervals (ITI) for nicotine shorter than those for quinine, again suggesting that these tastants were discriminated.

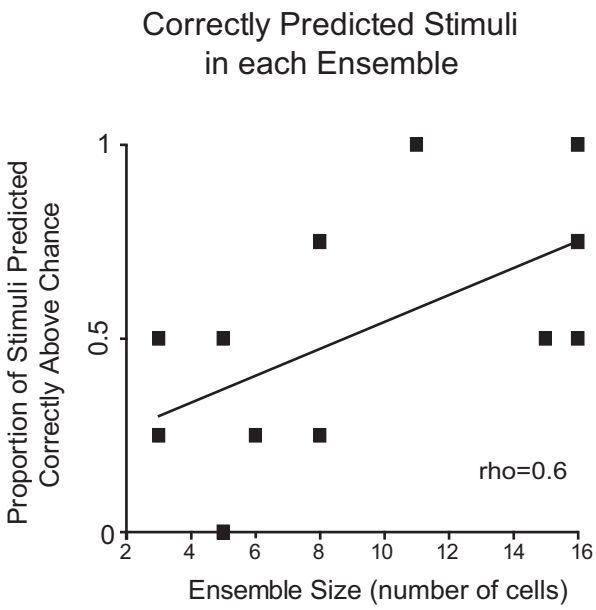

Fig. S7. Neural ensemble size correlates with efficacy in predicting stimulus identity. For each stimulus, the percentage of correct predictions were calculated separately for each ensemble and compared with chance level in that ensemble (25 or 50%). The proportion of tested stimuli that were predicted above chance by each ensemble correlated positively with ensemble size (Spearman rho $= 0.6$, $P < 0.04$).

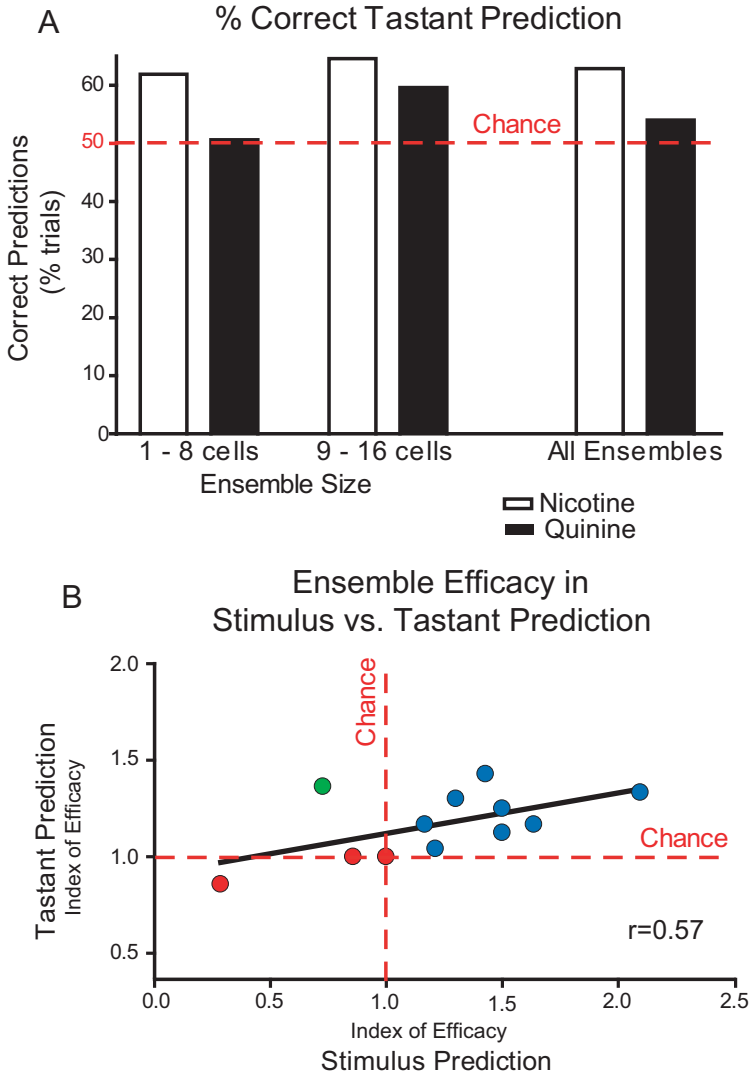

Fig. S8. GC ensemble stimulus and tastant predictions. (*A*) To confirm the importance of ensemble size in tastant discrimination, ensembles were divided into 2 groups: small ensembles, with the same or less than half the neurons in the largest ensemble (3–8 neurons, 5.4 neurons on average, 7 ensembles), and large ensembles, with more than half the neurons in the largest ensemble (9–16 neurons, 14.8 neurons on average, 5 ensembles). As expected (see Fig. S7), the percentage of correct tastant predictions was higher in large ensembles (64.6% for nicotine and 59.6% for quinine) than small ensembles (61.9% for nicotine and 50.6% for quinine), particularly for quinine. (*B*) To test if ensemble efficacy in stimulus and tastant prediction are positively correlated, we defined a homogeneous index of prediction efficacy. To that effect, for each ensemble, the overall stimulus and tastant prediction accuracy were calculated (i.e., total number of correct predictions across all stimuli or tastants was divided by the total number of observations for that ensemble) and then normalized by the respective stimulus (25 or 50%) or tastant (50%) prediction chance level. The resulting indexes of stimulus or tastant prediction efficacy are on a continuous scale of positive values, with those >1 reflecting above chance prediction levels. In the figure, dashed red lines were drawn at prediction efficacies of 1. Eight ensembles were predictive of both stimulus and tastant identity (blue circles) whereas 3 others were at or below chance levels in both cases (red circles). Only one ensemble (green circle) had dissonant prediction efficacies because it was predictive of tastant, but not stimulus, identity. Because we hypothesized that ensembles containing more information about stimulus identity would also be the more predictive of tastant identity, Pearson's correlation coefficient between the 2 indexes was calculated and its' significance was tested on a 1-tailed distribution. Indeed, the indexes were shown to correlate significantly ($r = 0.57$, $P < 0.03$, 1-tailed).

Table S1. Preference for quinine and nicotine measured under several behavioral paradigms in rats and mice (TRPM5 KO and WT)

Subject	Test	Test stimulus (mM)	Reference stimulus (mM)	Preference Ratio/Index (mean ± SEM)	*P* value (1 sample *t* test vs. 0.5 or 1)	SequentialBonferroni correction (Holm's)
Rats	Two-bottle	Nicotine (0.03)	Water	0.52 ± 0.09	0.86	$P < 0.05$
		Nicotine (0.1)		0.56 ± 0.08	0.41	
		Nicotine (0.3)		0.41 ± 0.05	0.11	
		Nicotine (1)		0.16 ± 0.05	**$P < 0.0001$**	$P < 0.0125$
		Nicotine (3)		0.02 ± 0.005		$P < 0.01$
		Nicotine (10)		0.03 ± 0.01		$P < 0.0083$
Rats	Two-bottle	Quinine (0.01)	Water	0.46 ± 0.07	0.54	$P < 0.05$
		Quinine (0.03)		0.4 ± 0.07	0.17	
		Quinine (0.1)		0.34 ± 0.04	**0.0031**	$P < 0.017$
		Quinine (0.3)		0.13 ± 0.03	**$P < 0.0001$**	$P < 0.0125$
		Quinine (1)		0.07 ± 0.01		$P < 0.01$
		Quinine (3)		0.01 ± 0.007		$P < 0.0083$
KO mice	Two-bottle	Quinine (10)	Water	0.54 ± 0.05	0.45	$P < 0.05$
WT mice				0.02 ± 0.006	**$P < 0.0001$**	$P < 0.025$
KO mice	Two-bottle	Nicotine (1)	Water	0.16 ± 0.04	**$P < 0.0001$**	$P < 0.05$
WT mice				0.19 ± 0.05		$P < 0.025$
KO mice			Quinine (10)	0.14 ± 0.05		$P < 0.017$
WT mice				0.93 ± 0.03		$P < 0.0125$
KO mice	Two-bottle	Nicotine (0.5)	Water	0.25 ± 0.05	**0.0007**	$P < 0.017$
WT mice				0.38 ± 0.05	**0.031**	$P < 0.05$
KO mice			Quinine (10)	0.17 ± 0.07	**0.0016**	$P < 0.025$
WT mice				0.9 ± 0.04	**$P < 0.0001$**	$P < 0.0125$
KO mice	Brief access	Nicotine (0.5)	Water	0.98 ± 0.05	0.71	$P < 0.05$
		Nicotine (1)		0.86 ± 0.04	**0.001**	$P < 0.01$
		Quinine (10)		0.93 ± 0.05	0.2	$P < 0.05$
WT mice		Nicotine (0.5)		0.92 ± 0.05	0.15	
		Nicotine (1)		0.83 ± 0.05	**0.012**	$P < 0.0125$
		Quinine (10)		0.51 ± 0.04	**$P < 0.0001$**	$P < 0.0083$
KO mice	Two-bottle	Nicotine (0.5)	Water	0.25 ± 0.05	**0.0007**	$P < 0.0125$
		Nicotine (1)		0.22 ± 0.07	**0.0035**	$P < 0.017$
Caps-tx*		Nicotine (0.5)		0.25 ± 0.07	**0.0029**	$P < 0.025$
		Nicotine (1)		0.3 ± 0.08	**0.039**	$P < 0.05$
KO mice	Two-bottle	Capsaicin (0.01)	1% DMSO	0.17 ± 0.06	**0.0004**	$P < 0.025$
Caps-tx*				0.38 ± 0.05	**0.037**	$P < 0.05$
WT mice	Brief access	Nicotine (0.5)	Water	0.94 ± 0.04	0.15	$P < 0.05$
		Nicotine (1)		0.82 ± 0.06	0.014	$P < 0.01$
		Nicotine (3)		0.79 ± 0.06	**0.0041**	$P < 0.0083$
		Nicotine (0.5)	Mecamylamine (0.3)	0.92 ± 0.07	0.32	$P < 0.05$
		Nicotine (1)		1.01 ± 0.07	0.88	
		Nicotine (3)		0.94 ± 0.07	0.4	
KO mice	Brief access	Nicotine (0.5)	Water	0.92 ± 0.06	0.23	$P < 0.01$
		Nicotine (1)		0.99 ± 0.07	0.9	$P < 0.05$
		Nicotine (3)		0.68 ± 0.08	**0.0051**	$P < 0.0083$
		Nicotine (0.5)	Mecamylamine (0.3)	1.02 ± 0.07	0.79	$P < 0.05$
		Nicotine (1)		1.04 ± 0.08	0.59	
		Nicotine (3)		0.94 ± 0.06	0.28	
WT mice	Brief access	Mecamy. (0.3)	Water	1.04 ± 0.08	0.63	$P < 0.05$
		Quinine (10)		0.43 ± 0.06	**$P < 0.0001$**	$P < 0.025$
		Qui/Mec (10/.3)		0.46 ± 0.08	**$P < 0.0001$**	$P < 0.017$

Significant *P* values after sequential Bonferroni correction for multiple comparisons using Holm's method [(1979) *Scand J Statistics* 6:65–70] (applicable for non-independent measures) are underlined and in bold. *, Capsaicin treated KO animals.

Other Supporting Information Files

SI Appendix