LETTER

doi:10.1038/nature15763

Sweet and bitter taste in the brain of awake behaving animals

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Taste is responsible for evaluating the nutritious content of food, guiding essential appetitive behaviours, preventing the ingestion of toxic substances, and helping to ensure the maintenance of a healthy diet. Sweet and bitter are two of the most salient sensory percepts for humans and other animals; sweet taste allows the identification of energy-rich nutrients whereas bitter warns against the intake of potentially noxious chemicals[1]. In mammals, information from taste receptor cells in the tongue is transmitted through multiple neural stations to the primary gustatory cortex in the brain[2]. Recent imaging studies have shown that sweet and bitter are represented in the primary gustatory cortex by neurons organized in a spatial map[3,4], with each taste quality encoded by distinct cortical fields[4]. Here we demonstrate that by manipulating the brain fields representing sweet and bitter taste we directly control an animal's internal representation, sensory perception, and behavioural actions. These results substantiate the segregation of taste qualities in the cortex, expose the innate nature of appetitive and aversive taste responses, and illustrate the ability of gustatory cortex to recapitulate complex behaviours in the absence of sensory input.

In mice, sweet and bitter activate cortical fields in the insula (taste cortex) that are separated topographically by approximately 2 mm (ref. 4) (Fig. 1a and Extended Data Fig. 1). We hypothesized that if these cortical fields represent sweet and bitter percepts, their direct activation would evoke 'bitter and sweet sensation' even in the absence of an actual bitter or sweet stimulus. To optogenetically control activation of the gustatory cortex, we introduced channelrhodopsin[5] (ChR2) to the insula of wild-type mice by stereotaxic injection of adeno-associated virus (AAV) targeted to either the bitter or the sweet cortical field (see Fig. 1a, b, Extended Data Fig. 1, Supplementary Table 1 and Methods for details). Single unit recordings of the insular cortex of transduced animals demonstrated that photostimulation evoked reliable neuronal firing that was phase locked to light delivery (Fig. 1c and Extended Data Fig. 1b).

We reasoned that optogenetic activation of the sweet cortical field should trigger behavioural attraction, whereas stimulation of the bitter field should cause strong behavioural avoidance. We used a place-preference test[6] where animals expressing ChR2 in the sweet cortex were introduced to a two-chamber arena in which presence in one of the two chambers was coupled to optogenetic stimulation, in the absence of any reward or punishment; we then determined the animal's preference index as a measure of the time spent in the chamber that was coupled with light stimulation. When the sweet cortical field was stimulated, animals developed strong preference for the chamber coupled to ChR2 stimulation (Fig. 1d and Extended Data Fig. 2). This preference could be transferred to either side of the arena by switching the chamber coupled to the laser stimulation of sweet cortex (Fig. 1d, compare chamber 1 versus chamber 2). When the same sets of experiments were performed in animals expressing ChR2 in the bitter cortical field, mice now displayed a range of unconditioned aversive behaviours (see next section), and after just a few sessions strongly avoided the chamber linked to photostimulation (Fig. 1e). Mice injected with a control AAV expressing enhanced green fluorescent protein (AAV–eGFP construct) exhibited no significant place preference after laser stimulation of either the sweet or bitter cortical fields (Extended Data Fig. 2b). Together, these observations demonstrate that neurons in the sweet and bitter cortical fields drive attractive and aversive responses, respectively.

Next, we examined if activation of the bitter and sweet cortical fields evokes classical taste behaviours[7]. We hypothesized that optogenetic activation of the bitter cortical field should trigger strong light-dependent suppression of licking, while activation of the sweet cortical field should trigger appetitive responses.

We used a behavioural test where motivated animals (thirsty) were trained to lick water in response to a combination visual/tone cue in a head-restrained set-up[8] (see Methods). We then subjected the trained animals expressing ChR2 in the bitter cortical field to testing sessions consisting of a series of water-only trials, but in half of the trials the bitter cortical field was stimulated upon contact of the tongue with the water spout.

During the entire session we imaged (facial features), recorded, and measured licking responses. Figure 2 demonstrates that when the bitter cortical field was stimulated, there was a dramatic suppression of licking behaviour (see also Supplementary Video 1), with the animal's response closely following the ChR2 activation of the bitter cortex. Notably, after strong laser stimulation (10–20 mW), the animals displayed prototypical taste rejection orofacial responses, sometimes including gagging (gaping[9]), and attempts to clean and rid the mouth of the non-existent bitter tastant (Supplementary Video 1; see legend for details).

What about the sweet cortical field? A characteristic feature of sweet taste is that non-thirsty animals remain robustly attracted to sweet solutions, even though they exhibit limited interest for water[10]. Therefore, we predicted that a mildly water-satiated animal expressing ChR2 in the sweet cortical field would still show little attraction for water in control trials (referred to as off-trials), but would exhibit significantly enhanced licking during water trials coupled to laser stimulation of the sweet cortical field (referred to as on-trials). Importantly, the experiment was set up such that the laser shutter was under contact-licking operation, so the animal had control of its own stimulation during the on-trials, and therefore only persistent licking (self-stimulation) would continue to activate the sweet cortex. Our results demonstrate that animals aggressively self-stimulated during on-trial sessions, with ChR2 activation of the sweet cortical field radically increasing licking behaviour, even though the spout still delivered only water, as in the off-trials (Fig. 2b, d).

Just as a lot of sugar can 'mask' a bitter tastant, we hypothesized that strong activation of the cortical field representing sweet taste might be capable of overcoming the natural aversion to an orally applied bitter

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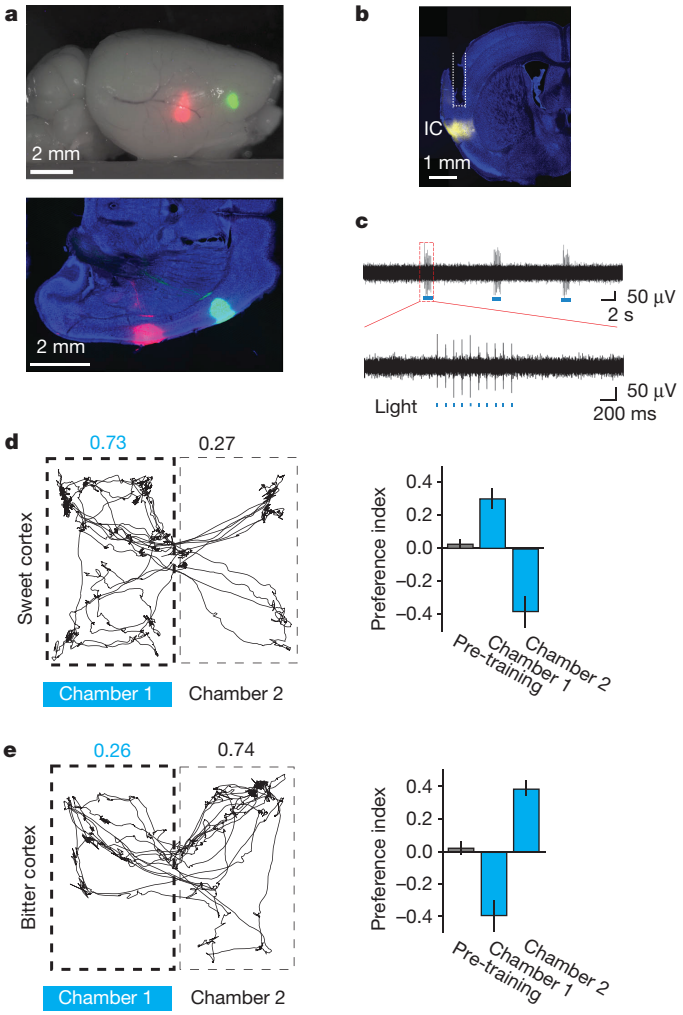

Figure 1 | Place preference by photostimulation of the sweet and bitter cortical fields. a, Sample injection of reporters in stereotactic coordinates defining the sweet and bitter cortical fields. Top: sweet cortex labelled with AAV–GFP and bitter cortex with AAV–TdTomato; bottom: a horizontal section. See Extended Data Fig. 1 for additional data. **b**, Coronal section of a mouse brain (bregma −0.2) stained with TO-PRO-3 (blue). Shown is a representative histological sample of the bitter cortical field expressing ChR2 fused to yellow fluorescent protein (ChR2–YFP), illustrating the location and trajectory (dotted lines) of the implanted guide cannula; IC, insular cortex. **c**, *In vivo* recording of ChR2-expressing insular cortical neurons in response to light stimulation (ten pulses, 10 Hz). The expanded traces show responses to each light pulse (blue bars below the trace). **d**, Left: representative tracking of a mouse during the 5 min preference test in a two-chamber arena; chamber 1 was coupled to light stimulation of the sweet cortical field during the training sessions. Shown are the fractions of time spent in each chamber. Right: quantitation of preference index before (pre-) and after (chamber 1) training with photostimulation of the sweet cortical field ($n = 13$ animals; Mann–Whitney *U*-test, $P < 0.003$). Preference can be readily reversed by light stimulation in the opposing side (chamber 2, $n = 6$; $P < 0.02$). **e**, Representative mouse track and quantitation of preference index in mice expressing ChR2 in the bitter cortical field; note significant aversion to the chamber coupled to photostimulation (chamber 1, $n = 15$; Mann–Whitney *U*-test, $P < 0.005$); this behavioural aversion can be switched to the opposite chamber by re-exposure to photostimulation in chamber 2 ($n = 4$; $P < 0.03$). Values are mean ± s.e.m. See Extended Data Fig. 2b for GFP control injections.

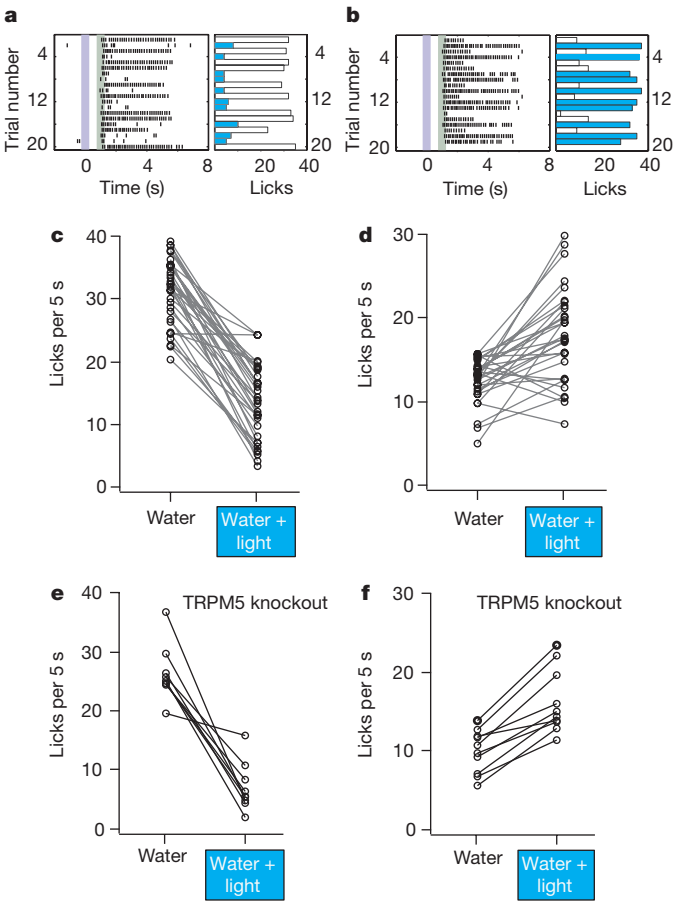

Figure 2 | Photostimulation of bitter and sweet cortical fields drives aversive and appetitive behaviours. a, **b**, Representative raster plots (left) and histograms (right) illustrating licking events during a 5 s licking window in the presence (blue) or absence (open) of light stimulation of (**a**) the bitter and (**b**) the sweet cortical fields. The purple line at time zero indicates the start of each trial; the green line indicates the onset of water delivery. **c**, **d**, Quantitation of licking responses with and without light stimulation in (**c**) the bitter cortical field ($n = 34$, Mann–Whitney *U*-test, $P < 4 \times 10^{-12}$) or (**d**) sweet cortical field ($n = 31$, Mann–Whitney *U*-test, $P < 5 \times 10^{-5}$) of wild-type mice. **e**, **f**, Quantitation of licking responses in TRPM5 knockout mice (**e**, bitter cortical fields, $n = 9$, Mann–Whitney *U*-test, $P < 5 \times 10^{-5}$; **f**, sweet cortical fields, $n = 10$, Mann–Whitney *U*-test, $P = 0.001$). Each point indicates data from an individual mouse before and after photostimulation.

stimulus. Therefore, we asked whether photostimulation of the sweet cortical field in animals expressing ChR2 in sweet cortex could switch preference for an otherwise aversive tastant. Conversely, we also tested whether photostimulation of the bitter cortical field triggers aversion to an otherwise sweet, attractive tasting chemical. Our results (Extended Data Fig. 3) show both postulates to be correct, and highlight how activation of selective taste cortical fields can mask the hedonic value of oral taste stimulation.

The experiments described above show that direct control of primary taste cortex can evoke specific, reliable, and robust behaviours naturally symbolic of taste responses to chemical tastants. These gain-of-function studies also illustrate how top-down control of the taste pathway can activate innate, immediate responses representing sweet and bitter taste.

To formally demonstrate that these cortically triggered behaviours are innate (that is, independent of learning or experience) we performed similar stimulation experiments in mice that had never tasted sweet or bitter chemicals (TRPM5 null mice[10]; Extended Data Fig. 4). Indeed, our results (Fig. 2e, f) showed that even in animals that had never experienced sweet or bitter taste, ChR2 activation of the corresponding cortical fields still triggered the appropriate behavioural response, thus substantiating the predetermined nature of the sense of taste.

It has been known for a long time that decerebrated animals can still exhibit stereotyped attraction and aversion to sweet and bitter chemicals[11]. This is thought to be mediated by brainstem taste circuits dedicated to immediate responses[11,12]. Therefore, to evaluate the necessity (and sufficiency, see next section) of taste cortex in taste

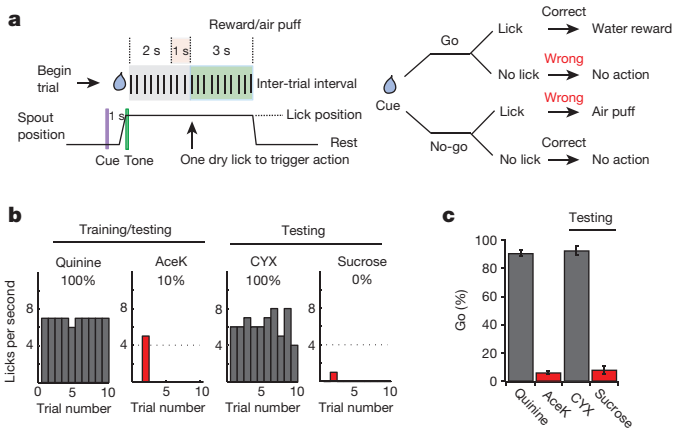

Figure 3 | Go/no-go taste discrimination task in head-restrained mice. **a**, Schematic and flow chart of the go/no-go taste discrimination task. Each trial starts with a visual cue (purple line), followed 1 s later by a tone (green line) to alert mice to initiate licking. After sampling, mice were given 3 s to continue to lick (go) or withhold licking (no-go) in response to the test tastant. For go trials, mice were rewarded with water (3 s) if they chose to lick within the 3-s interval. For no-go trials, mice received a mild air puff to the eyelid if they failed to withhold licking. After the reward/penalty phase, the spout retracted and was cleared for the next trial; inter-trial intervals were 8 s. **b**, Representative histograms illustrating recognition and generalization within bitters and sweets. This animal was trained and tested with 4 mM AceK (sweet no-go) and 0.5 mM quinine (bitter go), and then assayed with 100 mM sucrose and 10 μM cycloheximide (CYX). **c**, Quantitation in nine animals, demonstrating highly reliable taste recognition and discrimination. Values are mean ± s.e.m.

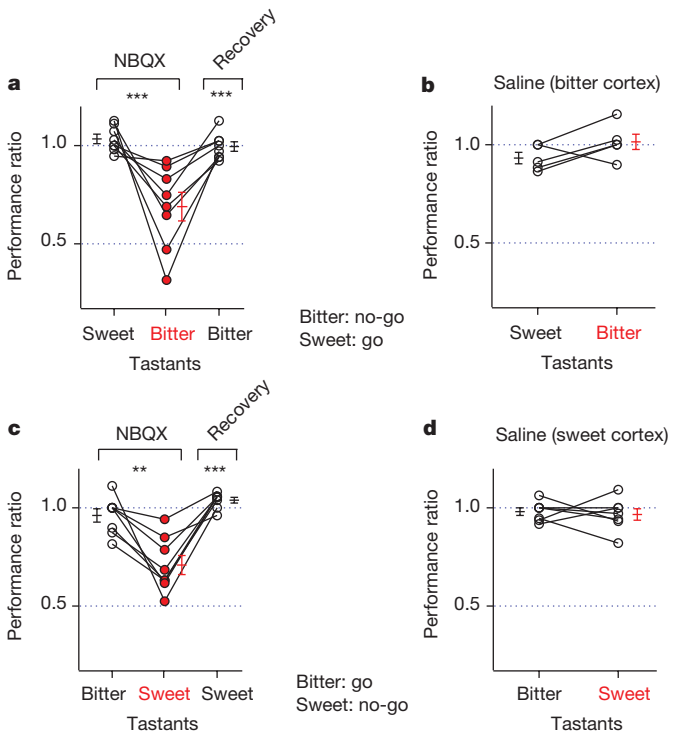

Figure 4 | Inactivation of the bitter and sweet cortical fields disrupts taste discrimination. **a**, Quantitation of performance ratios (see methods) before and after bilateral silencing (NBQX, 5 mg ml^{-1}) of the bitter cortical fields ($n = 8$); animals were trained to no-go to bitter and go to sweet. Note the impact in bitter taste discrimination, but no significant effect in sweet taste. After washout of the drug, the animal's ability to recognize bitter is restored. Comparable results are obtained when animals are instead trained to go to bitter and no-go to sweet (Extended Data Fig. 2). **b**, Quantitation of performance ratios with saline controls in bitter cortical fields; there is no significant effect on sweet or bitter taste ($n = 5$; Mann–Whitney U-test, $P = 0.14$). **c**, Quantitation of performance ratios with bilateral injection of NBQX in the sweet cortical fields ($n = 8$). Animals were trained to no-go to sweet and go to bitter; note significant deficit in sweet taste, but no effect on bitter taste. After washout of the drug, the animal's ability to recognize sweet is restored. **d**, Saline injections in the sweet cortical fields have no significant effect on bitter or sweet taste ($n = 7$; Mann–Whitney U-test, $P = 0.80$). Values are mean ± s.e.m. Mann–Whitney U-test, $^{**}P < 0.01$, $^{***}P < 0.001$.

recognition and discrimination, we needed to design a test that bypasses immediate taste responses, and instead engages cortical circuits. In this assay (go or no-go behavioural test)[13,14], thirsty animals were trained to sample a test tastant from a spout, and then to report its identity either by licking (go) or withholding licking (no-go) (Fig. 3). This learned behaviour required the animal to sample the cue, recognize the tastant, and execute the appropriate behaviour in each trial. We trained animals several ways, including to go to bitter and no-go to sweet, exactly the opposite of the innate drive. After 10–15 sessions of training (each consisting of 80 trials, with 40 randomly presented sweet and 40 bitter cues), mice were able to report the tastant's identity with almost 90% accuracy (Fig. 3). To further demonstrate the selectivity of the assay and responses, we next tested the animals with sweet and bitter chemicals not used in the training phase. Given that all sweet tastants activate the same sweet taste receptor[15–17], and all bitters the same class of taste receptor cells[18], we expected that novel sweets should also be recognized as no-go cues, whereas novel bitters should be seen as go cues. Indeed, animals trained with the bitter tastant quinine and the artificial sweetener acesulfame K (AceK) recognized and responded with similar accuracy to cycloheximide and sucrose, bitter and sweet tastants with completely different chemical structures from the training set (Fig. 3c).

We implanted cannulae bilaterally into the bitter cortical fields of trained animals (Supplementary Table 1), waited 2 weeks for recovery, and assayed tastant discrimination in the go/no-go behavioural test before and after bilateral injection of a glutamate receptor antagonist (NBQX) to silence cortical activity[19,20]. As shown in Fig. 4, silencing the bitter cortical fields prevented animals from reliably identifying the bitter tastant (see Extended Data Fig. 5 for additional examples using the reverse training test). In contrast, their ability to recognize sweet tastants remained unimpaired. Importantly, the loss of bitter taste function was fully reversible upon washout of the drug (Fig. 4a), whereas injection of a saline control in the bitter cortical fields had no significant effect on either bitter or sweet taste sensing (Fig. 4b). We used the same strategy to conduct loss-of-function experiments in the sweet cortex. Indeed, bilateral silencing of the sweet cortical fields disrupted sweet, but not bitter, taste discrimination (Fig. 4c, d). As expected, animals recovered sweet taste perception after drug washout. Taken together, these results substantiate the essential role of the sweet and bitter cortical fields in sweet and bitter taste recognition.

What is the mouse sensing upon direct activation of a taste cortical field? Does optogenetic stimulation create internal representations that mimic those evoked by sweet and bitter chemicals on the tongue? If so, we reasoned that animals trained to recognize and report the sensory features of an orally provided sweet or bitter tastant (for example, in a go/no-go assay) should respond similarly to optogenetic stimulation of the corresponding cortical fields, even though the animal had never been trained with light stimulation. In essence, if light and the chemical tastant evoke similar percepts, then light will generalize to the learned responses associated with the orally supplied stimulus.

We first focused on sweet, because activation of the bitter cortical field evokes prototypical and highly salient orofacial responses that are already strongly indicative of bitter perception (Supplementary Video 1). We introduced ChR2 into the sweet cortical field of untrained mice and validated robust light-triggered appetitive responses (see Fig. 2). Then, the mice were trained in a go/no-go behavioural test where they learned to associate go with a bitter chemical and a low-salt solution (Fig. 5a), and no-go with sweet taste. Critically, under this test, mice needed to report both an aversive (bitter) and an attractive cue (low salt, see also Extended Data Fig. 6) in the same arm of the behavioural

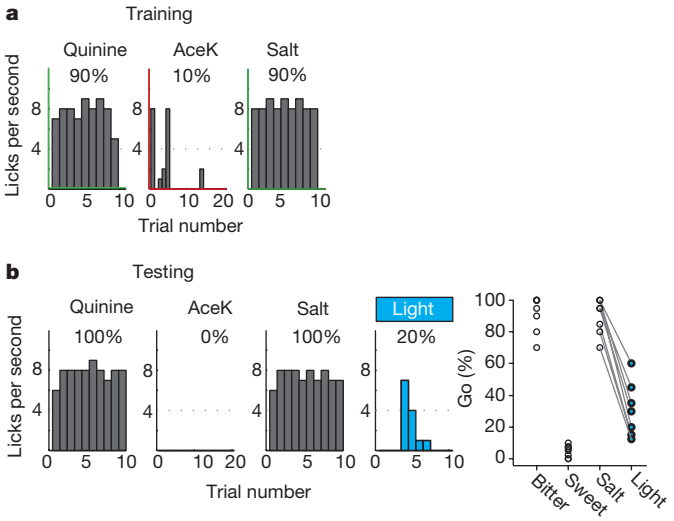

Figure 5 | Cross-generalization between orally supplied taste stimuli and photostimulation of the sweet cortex. a, Representative histograms illustrating mouse performance during a training session in the go/no-go discrimination task. The mouse was trained to go to bitter (0.5 mM quinine) and low salt (20 mM NaCl), and no-go to sweet (4 mM AceK). Note that both bitter (aversive) and low salt (attractive) were used in the same branch of the behavioural task (go) to exclude the valence as an identifier. **b**, Left: representative histograms illustrating cross-generalization between taste stimulation and photostimulation of the sweet cortical field. Right: quantitation of the responses from individual animals to quinine, AceK, salt and salt + light ($n = 8$, Mann–Whitney U-test, $P < 0.0002$).

test, hence removing pure valence[21] as a way of identifying tastants. After mice performed at or above 80% accuracy (Fig. 5a), we assayed whether light (previously triggering strong appetitive responses) was being sensed and reported as sweet (now a no-go response). Animals were tested with 50 randomized trials consisting of 20 bitter, 10 sweet, 10 low salt, and 10 low salt linked to light stimulation of the sweet cortical field. Our results (Fig. 5b) showed that light stimulation of sweet cortex was indeed being sensed as a 'fictive' sweet stimulus, eliciting strong and reliable no-go responses; Extended Data Fig. 7 shows similar experiments and equivalent findings with bitter cortex. Taken together, these results show that activation of a taste cortical field recapitulates an internal representation (for example, perceptual quality) naturally indicative of the orally presented chemical.

The essential role of the sense of taste is to evaluate the quality of a food source or a meal, and to activate the appropriate behavioural actions to consume or reject ingestion[1]. The taste cortex is thought to represent the basic sensory features of the different taste qualities[22,23], and to function as a central neural 'hub' that informs and integrates with other brain areas, and the internal state, to guide taste-dependent actions.

This work centred on the study of the two most distinctive taste qualities, sweet and bitter. These two differ not only in quality but also in valence, mediating innately attractive and aversive behaviours. Many studies have used optogenetics to activate ensembles of neurons and examine their physiological and behavioural consequences[6,24–27]. In this work we explored the internal representation of arguably the two most recognizable chemosensory percepts. Our current studies demonstrate that it is possible to govern an animal's perception and behavioural responses by direct manipulation of selective taste cortical fields. Notably, unlike our other fundamental chemical sense (smell), activation of the sweet and bitter cortical fields evokes predetermined behavioural programs, independent of learning and experience, further illustrating the hardwired and innate nature of the sense of taste.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 7 April; accepted 29 September 2015.
Published online 18 November 2015.

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Supplementary Information is available in the online version of the paper.

Acknowledgements We particularly thank H. Fischman and R. Lessard for suggestions, and members of the Zuker laboratory for comments. We also thank D. Salzman, K. Scott, and R. Axel for discussions. This research was supported in part by a grant from the National Institute of Drug Abuse (DA035025) to C.S.Z., and the Intramural Research Program of the National Institutes of Health, National Institute of Dental and Craniofacial Research (to N.J.P.R.). C.S.Z. is an investigator of the Howard Hughes Medical Institute and a Senior Fellow at Janelia Farms Research Campus, Howard Hughes Medical Institute.

Author Contributions Y.P. designed the study, performed experiments, and analysed data; S.G.-S. performed animals studies, viral injections, histology and analysed data; H.J. performed c-Fos expression studies; D.T. developed the initial behavioural platforms; N.J.P.R. and C.S.Z. designed the study, analysed data, and together with Y.P. wrote the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to C.S.Z. (cz2195@cumc.columbia.edu) or N.J.P.R. (nick.ryba@nih.gov).

METHODS

Stereotaxic injections and anatomy. All procedures were performed according to the approved protocols at Columbia University. Six- to eight-week-old C57BL6/J and *Trpm5*$^{-/-}$ mice were used for viral injections. All surgeries were performed using aseptic technique. Mice were anaesthetized with ketamine and xylazine (100 mg/kg body weight and 10 mg/kg body weight, intraperitoneal), placed into a stereotaxic frame, and unilaterally injected with ~30 nl AAV carrying ChR2 (AAV9.CamKIIa.hChR2(H134R)-EYFP.WPRE.SV40, Penn Vector Core) either in the sweet cortical field (bregma 1.6 mm; lateral 3.1 mm; ventral 1.8 mm), or the bitter cortical field (bregma −0.3 mm; lateral 4.2 mm; ventral 2.8 mm). After viral injection, a guide cannula (26 gauge, PlasticsOne) or a customized implantable fibre (200 μm, numerical aperture = 0.39) was implanted 300–500 μm above the injection site, and fixed in place with dental cement. A metal head-post was also attached and secured with dental cement for the purpose of head fixation during behavioural experiments. For pharmacological experiments, AAV–ChR2 was injected bilaterally in the sweet or bitter cortical fields, followed by bilateral implantation of guide cannulae. Mice were allowed to recover for 2–3 weeks before the start of behavioural experiments. Placements of viral injections, guide cannulae, and implantable fibres were histologically verified at the termination of the experiments by TO-PRO3 (1:1,000, Invitrogen) staining of coronal sections (100 μm). Fluorescent images were acquired using a confocal microscope (FV1000, Olympus).

Animals. All behavioural experiments with wild-type animals used 6- to 8-week-old male C57BL6/J mice. No statistical methods were used to predetermine sample size, and investigators were not blinded to group allocation. No method of randomization was used to determine how animals were allocated to experimental groups.

***In vivo* recordings.** Mice expressing ChR2 in taste cortex were anaesthetized with urethane (1.8 mg/g body weight), and the insular cortex was exposed as previously described[4]. Extracellular neural activity was recorded using a tungsten electrode (resistance 2.0–4.0 MΩ, FHC). Data were acquired, amplified, digitized, and bandpass filtered at 600–6,000 Hz with a Neuralynx data acquisition system. For photostimulation, 10 Hz, 5-ms pulses of 473 nm light (~5 mW) were delivered via a solid-state laser (Shanghai Laser & Optics Century Co.) coupled to an optical fibre (200 μm) positioned above the insular cortex.

c-Fos induction and Immunohistochemistry. Individual mice were implanted with an intraoral cannula[28] 3 days before c-Fos induction. On the day of experiments, mice were anaesthetized with urethane (1.6 mg/g body weight) and the trachea was cannulated to aid breathing during oral stimulus presentation. Tastants were perfused into the mouth through the intraoral cannula for 1.5 h at a rate of ~6 ml h^{-1}. Mice were allowed to rest for 30 min and processed for immunostaining as previously described. The brains were sectioned coronally at 100 μm, and labelled with goat anti-c-Fos (Santa Cruz, sc-52-G) overnight; Alexa 488 donkey anti-goat or cy3 donkey anti-goat (Jackson immunoResearch) were used to visualize c-Fos expression. All images were taken using an Olympus FluoView 1000 confocal microscope.

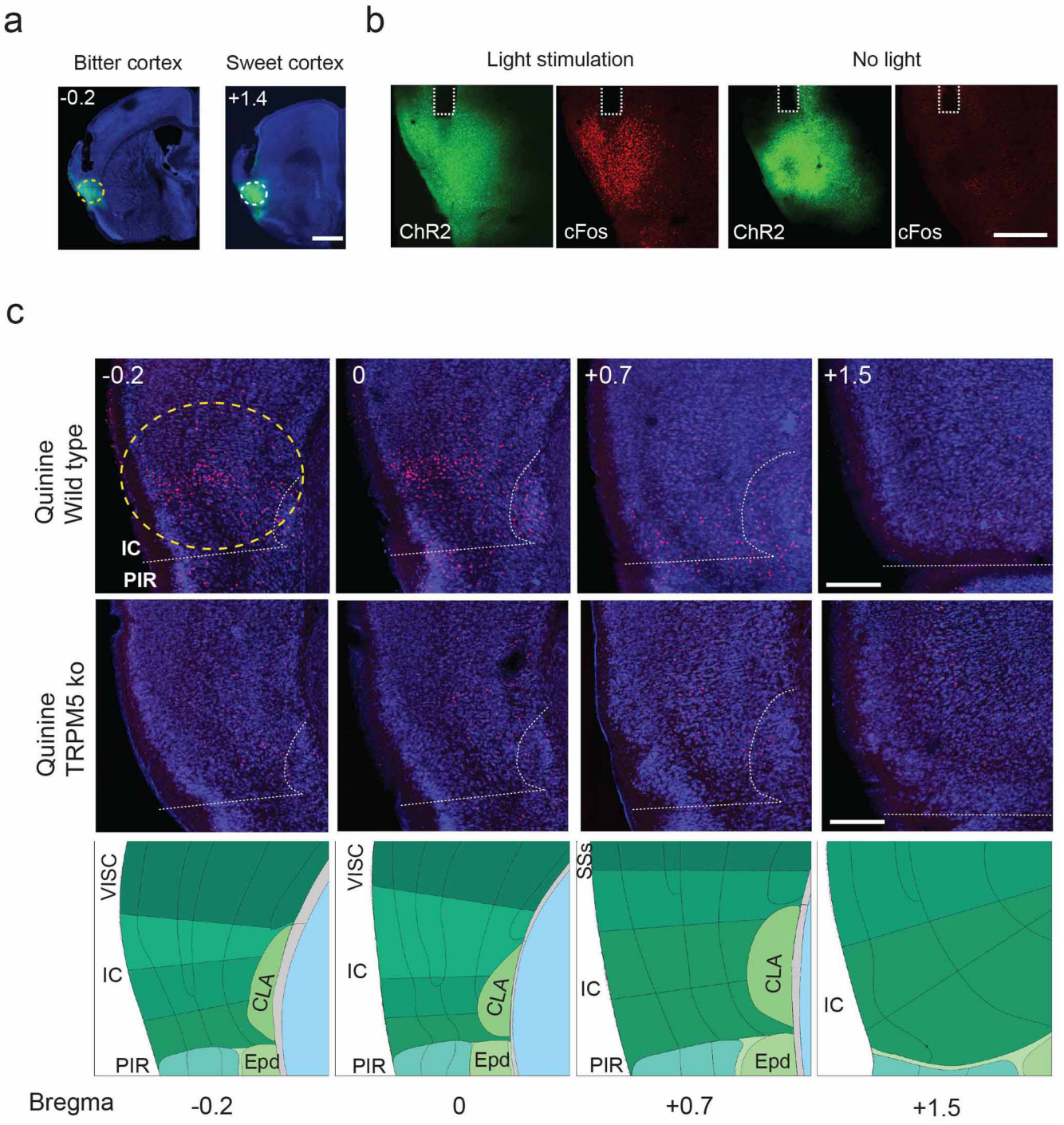
Place preference assays. Individual mice were tested in a custom-built two-chamber arena (30 cm × 30 cm total size). To differentiate the chambers, one chamber was designed with alternating black and white vertical stripes on its walls, whereas the other chamber was uniformly black. The arena was contained within a sound-attenuating cubicle (Med Associates). Mice were trained in the arena for 30 min with photostimulation of the sweet or bitter cortical field, and tested in the absence of any light stimulation for 5 min at the end of each session (defined as 'preference test'). Animal locations were tracked in real time by video imaging. At the beginning of the experiments, mice were acclimated to the arena for one session without light stimulation (defined as the pre-test condition). Photostimulation sessions began the next day, with two daily sessions for about 1 week. For each mouse, one chamber was randomly selected for photostimulation (chamber 1); when a mouse was located in this chamber, light was delivered (20 Hz, 20-ms pulses, 5–10 mW) for 5-s intervals, with 5-s rest periods to avoid over-stimulation or phototoxicity. After 1 week of sessions, a 'reverse probe' study was performed in a subset of animals, during which photostimulation was delivered in the opposing chamber (chamber 2). Animals were trained for a minimum of eight sessions, and the preference tests from the last three sessions were used to calculate the preference index (PI); $PI = (t_1 - t_2)/(t_1 + t_2)$, where t_1 is the fractional time a mouse spent in the chamber 1, and t_2 is the time spent in chamber 2.

Lick preference assays. Mice were first water-deprived for 24 h to motivate drinking behaviour. They were then introduced to head restraint and acclimated to drinking from a motor-positioned spout in 60-trial sessions (15 min), twice a day for 3 days. Each trial began with a flash, followed 1 s later by the spout swinging into position and a tone (4 kHz) to indicate the onset of water delivery. The spout remained in position for 5 s and was then removed. Mice were weighed daily during the habituation period as well as during any behavioural tests requiring water restriction. Additional water was supplied as necessary to ensure that animals maintained at least 85% of their initial body weight. To measure attractive/appetitive responses, mice were mildly water restrained (exhibiting an average of not more than 15 licks per 5-s trial in the lick preference assay), and supplied with approximately 5 μl water during each trial. To measure aversion, mice were water-deprived for 24 h, and supplied with approximately 10 μl water distributed over the full 5 s of spout presentation for each trial (so that animals remained eager to lick for all 5 s). To ensure animals were appropriately motivated in the lick preference behavioural assays (that is, thirsty to examine lick suppression, and mildly satiated to examine attraction), we examined animals exhibiting an average of at least 20 licks per 5 s trial as an indicator of 'thirst', and not more than 15 licks per 5 s trial for mild satiation. Animals were recorded by video for the entire session, and licks were analysed and counted by custom-written MATLAB software (Mathworks). Light stimulation and water delivery were controlled by the same software via an Arduino board. All animals analysed in these studies had histologically confirmed expression of ChR2 in the sweet or bitter cortical fields (Supplementary Table 1).

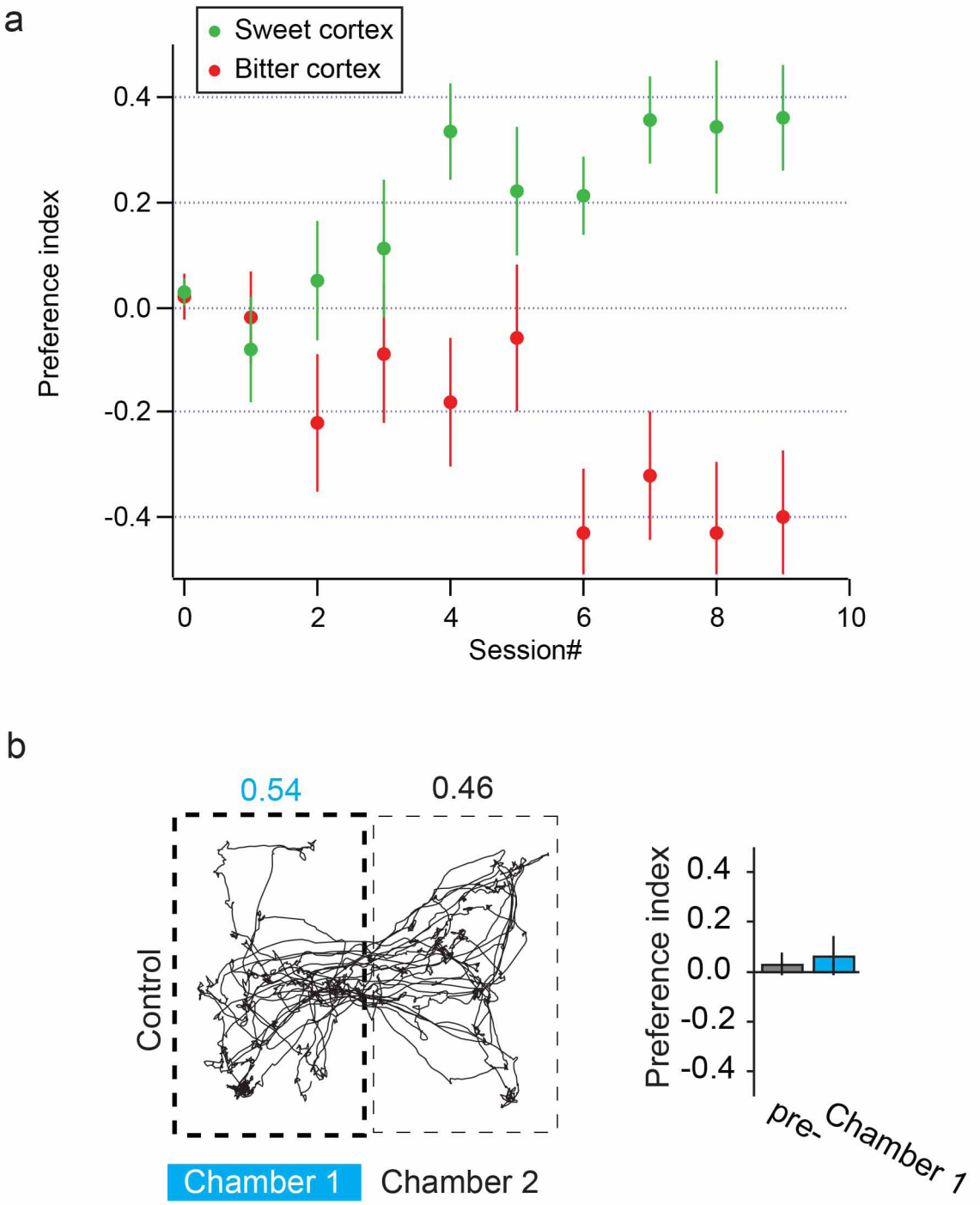
Go/no-go taste discrimination behaviour. Mice deprived of water for 24 h were first acclimated to consuming water in a head-restrained position for 15-min sessions over 2–3 days. Animals were then trained to perform a taste discrimination task, in which they were to lick, and receive a water reward, in response to a 2-μl presentation of tastant-1 ('go') and to withhold licking in response to tastant-2 ('no-go'). The presentation of the go and no-go stimuli was randomized. Each trial began with a visual cue (100-ms light flash), followed 1 s later by a tone (4 kHz, 300 ms) alerting the animal to sample the test tastant (for example, AceK or quinine; ~2 μl per sample). After sampling, mice were given 3 s either to continue to lick the spout (go trial) or to withhold licking (no-go trials). On go trials, if a mouse chose to lick within the 3-s interval, it was then rewarded with water for 3 s. On no-go trials, if a mouse failed to withhold licking within the 3-s interval, it was given a penalty of a gentle air puff to the eyelid. Mice were trained for two sessions per day, with 80 randomized trials (20 min) per session. For analysis, a 'go' response was defined as four or more licks in the second before reward or penalty. For photostimulation experiments, mice were first trained until they could effectively discriminate the tastants with ~90% accuracy (over 1–2 weeks). Then, on the 'probe' sessions, tastants and/or cortical photostimulation were presented during the sample period. Neither reward nor punishment was delivered for novel tastants or light stimulation. Before testing, animals with correctly placed cannulae were provisionally identified by ChR2 expression followed by one or two sessions of lick preference pre-tests. All animals analysed had histologically confirmed placement of cannulae and expression of ChR2 in the appropriate cortical field.

Pharmacological inhibition. Mice were trained to discriminate sweet from bitter in the go/no-go task with at least 90% accuracy. On the day of the experiment, mice were first tested with four taste stimuli (pre-test), including the original training tastants (2 mM AceK and 0.1 mM quinine) and a novel sweet and bitter tastant (50 mM sucrose and 2 μM cycloheximide). After the test, 0.3 μl of the glutamate receptor antagonist NBQX (5 mg ml^{-1} in 0.9% NaCl, Tocris Bioscience) was bilaterally infused into the chosen insular cortical fields over a period of 3 min. NBQX was delivered via an internal infusion needle inserted into the same guide cannulae used for light stimulation and connected to a 1-μl Hamilton syringe (PlasticsOne). Saline (0.9% NaCl) was used as control. After NBQX or saline infusion, animals were placed in their home cages to rest for 1.5 h. Mice were then re-tested with the same four taste stimuli on the go/no-go task (NBQX-test) and then at 8–24 h after rest (recovery-test). During tests, a water reward was given for correctly identifying the go cue, but no air puff was delivered for incorrectly identifying the no-go cue (to avoid possible re-learning). No reward or punishment was applied for the novel sweet and bitter tastants. A performance ratio was calculated for each taste quality: ratio = r_1/r_2, where r_1 is the percentage of correct responses during the NBQX-test or recovery-test, and r_2 is the percentage of correct responses during the pre-test. The percentage of correct responses for each taste quality was the average of go (%) for go taste stimuli (for example, quinine and cycloheximide), or the difference between (100 − go (%)) for no-go stimuli (for example, AceK and sucrose). All animals analysed had anatomically confirmed placement of cannulae in the appropriate cortical field. We note that we made several unsuccessful attempts to optogenetically silence the sweet and bitter cortical fields; this may have been due in part to the requirement for expression in most, if not all, relevant neurons.

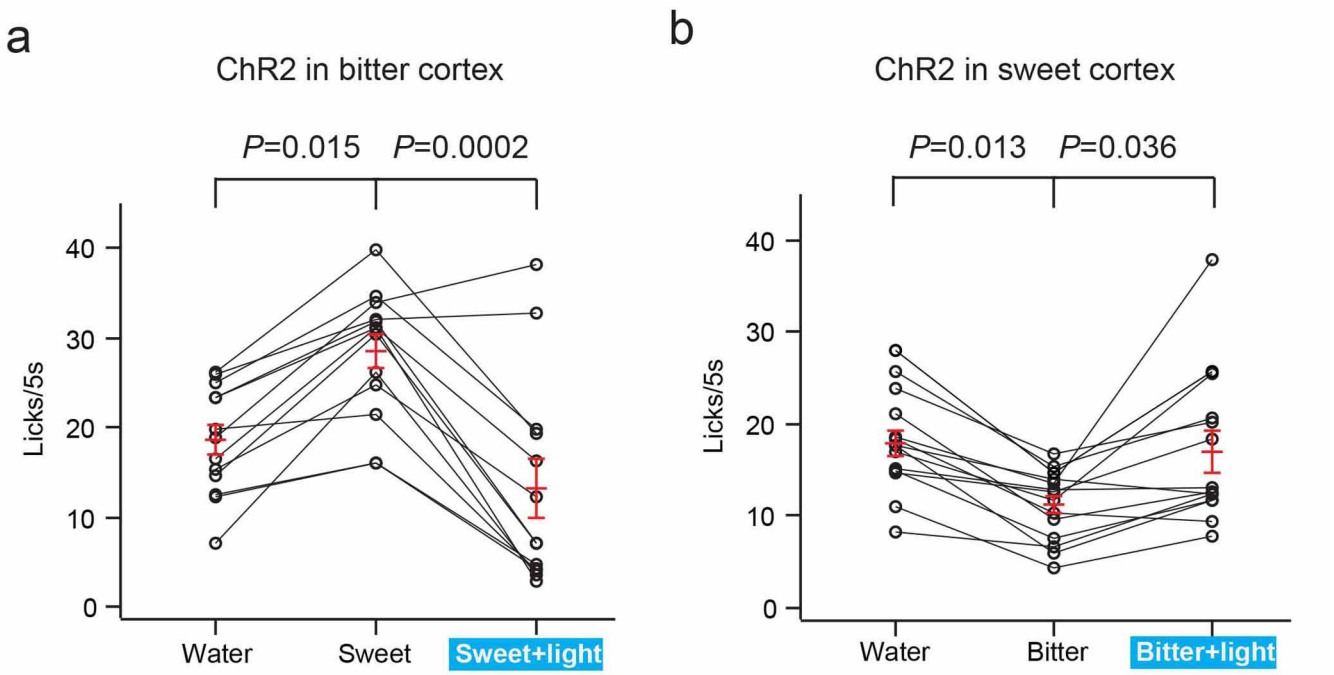
28. Tokita, K., Armstrong, W. E., St John, S. J. & Boughter, J. D. Jr. Activation of lateral hypothalamus-projecting parabrachial neurons by intraorally delivered gustatory stimuli. *Front. Neural Circuits* **8,** 86 (2014).



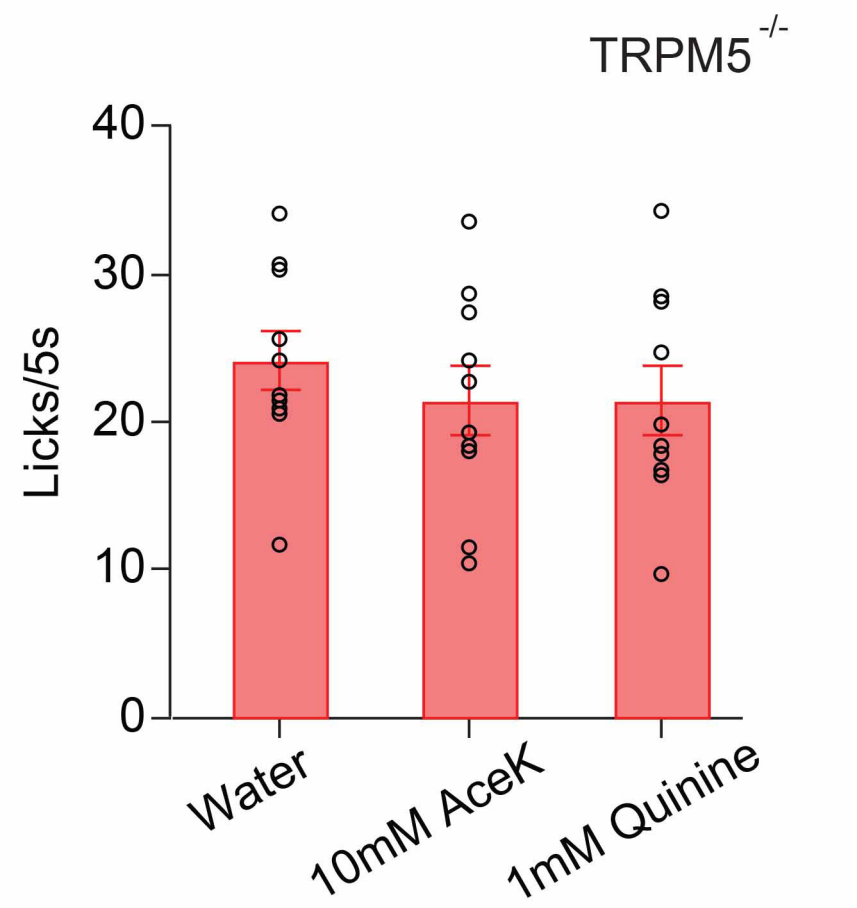
Extended Data Figure 1 | Expression of ChR2 in taste cortex. a, Samples of injection sites in the bitter and sweet cortical fields; shown are coronal sections (Fig. 1a shows a whole mount brain). ChR2–YFP expression (green), nuclei (blue; TO-PRO-3); numbers indicate position relative to bregma, and the dotted area highlight the location of the taste cortical fields (see **c**). **b**, Activation of insular neurons in sweet cortex triggers robust c-Fos expression; ChR2–YFP (green), c-Fos (red) after 10 min of *in vivo* photostimulation at 20 Hz, 20-ms pulses (5 s laser on, 5 s laser off, 5 mW). Dashed lines indicate the location of the stimulating cannulae/fibre. **c**, c-Fos (red) expression in bitter cortex (bregma 0, −0.2) after bitter tastant stimulation (10 mM quinine; see Methods for details). Note the absence of c-Fos expression in the middle (bregma +0.7) and sweet insular cortex (bregma +1.5). Importantly, specific labelling is abolished in taste blind animals (TRPM5 knockouts; middle row). The bottom row shows a diagram of the corresponding brain areas, adapted from the Allen Brain Atlas. Scale bars: 1 mm (**a**), 500 μm (**b**), 300 μm (**c**). PIR, piriform cortex; IC, insular cortex.



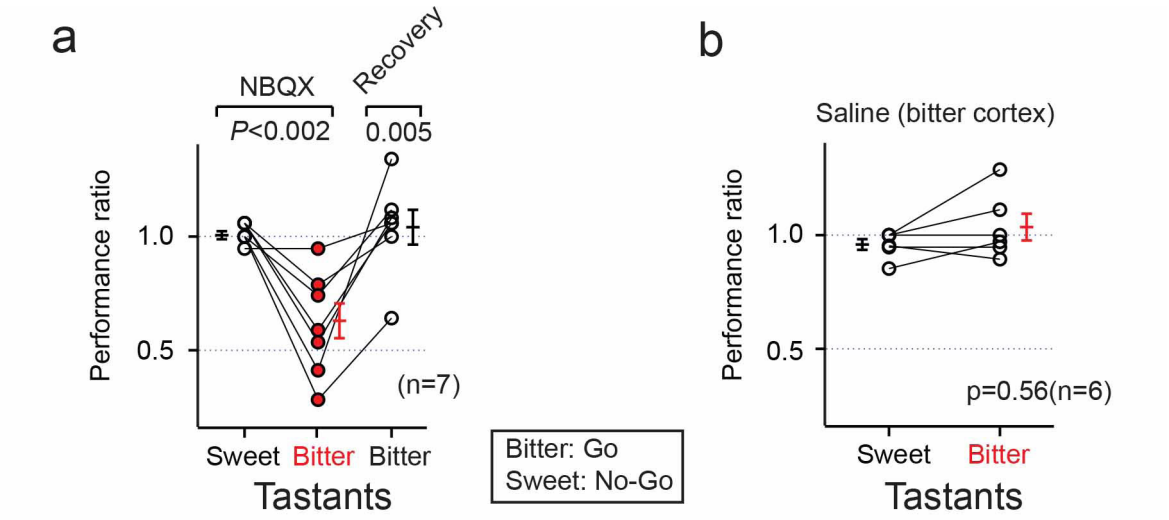
Extended Data Figure 2 | Acquisition of Place preference. a, The development of 'place preference' as a function of session number (each session was 30 min of training and 5 min of 'after-training' testing in the absence of light stimulation; $n = 13$ for sweet cortex, $n = 15$ for bitter cortex; see text and Methods for details). The average of sessions 6–8 was used in Fig. 1. Values are mean ± s.e.m. **b**, Representative mouse track and quantitation of preference index in control GFP-expressing mice; note no difference in preference between chambers ($n = 14$; Mann–Whitney U-test, $P = 0.74$). Values are mean ± s.e.m.



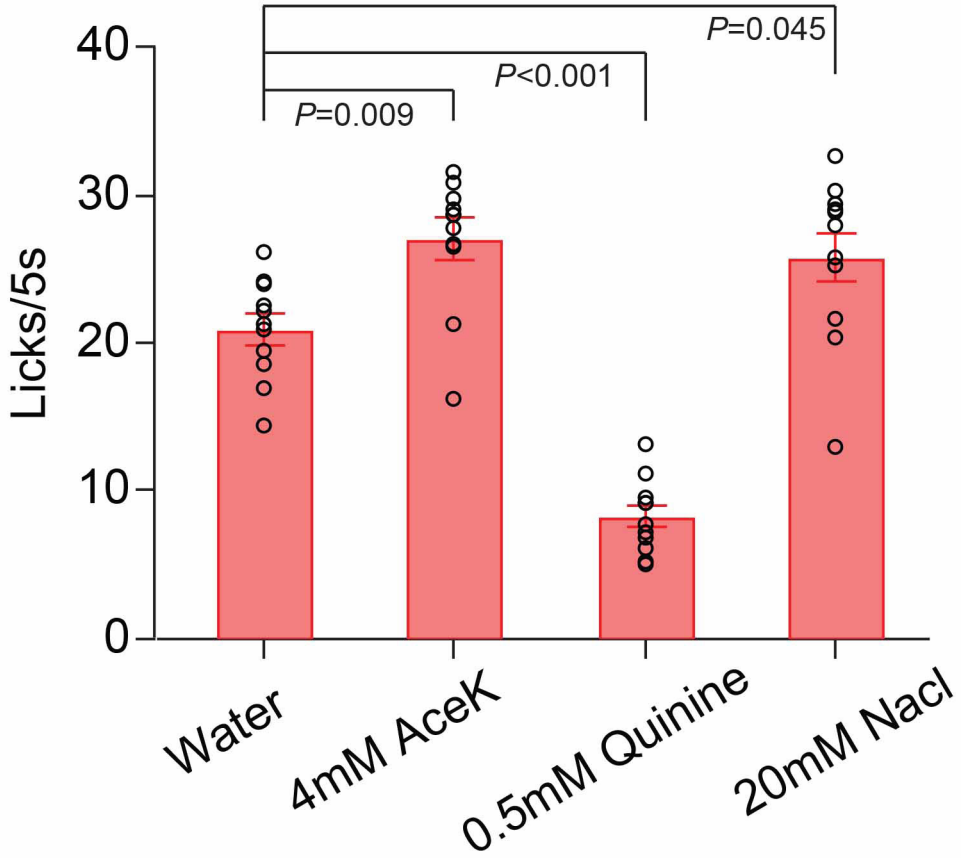
Extended Data Figure 3 | Photostimulation of insular cortical fields overcomes natural taste valence. a, Quantitation of licking responses in mice expressing ChR2 in the bitter cortical fields ($n = 13$, analysis of variance (ANOVA) test, Tukey's honest significant difference post hoc test). Photostimulation of the bitter cortical fields significantly suppress the natural attraction of the sweet tastant (4 mM AceK). **b**, Quantitation of licking responses in mice expressing ChR2 in the sweet cortical fields ($n = 14$, ANOVA test, Tukey's honest significant difference post hoc test). Photostimulation of the sweet cortical fields significantly overcomes the natural aversion of the bitter tastant (1 mM quinine). In both experiments, mice were water-restrained (but exhibited an average of not more than 30 licks per 5-s water trial) such that they were motivated to drink the bitter while showing attraction to sweet. Values are mean ± s.e.m.



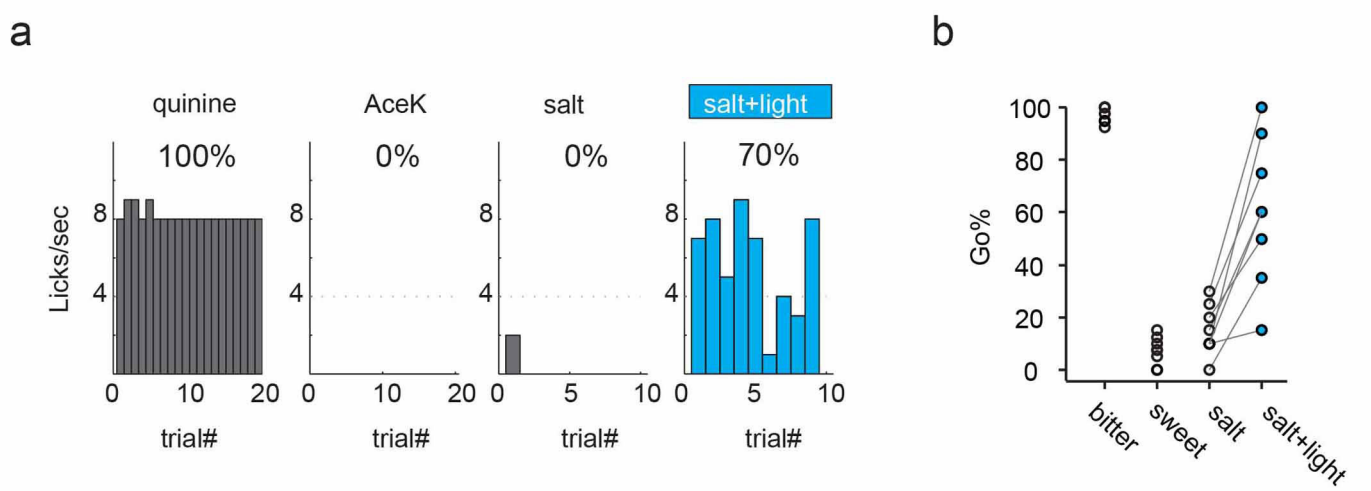
Extended Data Figure 4 | TRPM5 knockout mice do not taste sweet and bitter. Taste preference was tested in the head-restrained assay for wild type and TRPM5 homozygous mutants. Tastants were randomly delivered for a 5-s window (ten trials each). No significant difference was observed between water and sweet/bitter tastants in TRPM5 knockouts (ANOVA test, $P = 0.62$, $n = 10$; see ref. 10 for more details); circles indicate individual animals; bar graphs show mean ± s.e.m.



Extended Data Figure 5 | Inactivation of the bitter cortical fields in animals trained to go to bitter and no-go to sweet. a, Quantitation of performance ratios before and after bilateral silencing of the bitter cortical fields (NBQX, 5 mg ml^{-1}; $n = 7$) in animals trained to go to bitter and no-go to sweet. Note the impact in bitter taste discrimination, but no significant effect in sweet taste (Mann–Whitney *U*-test, $P < 0.002$). After washout of the drug, the animal's ability to recognize bitter is restored (Mann–Whitney *U*-test, $P < 0.005$). **b**, Quantitation of performance ratios with saline (0.9%) control in the bitter cortical fields ($n = 6$, Mann–Whitney *U*-test, $P = 0.56$). In both experiments, mice were trained with quinine and AceK, and tested with two pairs of sweet/bitter tastants (0.1 mM quinine and 2 mM AceK, 2 μM cycloheximide and 50 mM sucrose; see Methods for details).



Extended Data Figure 6 | Sweet and low salt are appetitive tastants. Taste preference was tested during a 10-min window using the head-restrained assay (see Methods for details). Four tastants were randomly delivered to animals for 5 s each (ten trials per tastant). Note that animals show significant attraction to sweet (AceK) and low salt (NaCl), but strong aversion to bitter ($n = 11$, ANOVA test, Tukey's honest significant difference post hoc test); circles indicate individual animals; bar graphs show mean ± s.e.m. These conditions were used in the experiments described in Fig. 5 and Extended Data Fig. 7.



Extended Data Figure 7 | Cross-generalization between orally supplied taste stimuli and photostimulation of the bitter cortex. a, Representative histograms illustrating cross-generalization between taste stimulation and photostimulation of the bitter cortical field. The mouse was trained to go to bitter (0.5 mM quinine) and no-go to sweet (4 mM AceK) and low salt (20 mM NaCl). **b**, Quantitation of the responses from individual animals to quinine, AceK, salt and salt + light ($n = 8$, Mann–Whitney U-test, $P < 0.002$). See also Fig. 4.