Innate liking and disgust reactions elicited by intraoral capsaicin in male mice

Ibin Han, Daisuke H. Tanaka*, Naofumi Uesaka*

Department of Cognitive Neurobiology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519 Japan.

*Correspondence to be sent to:
Daisuke H. Tanaka (for communication with the editorial office); Department of Cognitive Neurobiology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519 Japan; Tel +81-3-5803-5445; e-mail: dhtanaka.mphm@tmd.ac.jp

Naofumi Uesaka; Department of Cognitive Neurobiology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University (TMDU), 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519 Japan; Tel +81-3-5803-5445; e-mail: uesaka.cnb@tmd.ac.jp

Abstract

Liking and disgust are the primary positive and negative emotions, respectively, and are crucial for taking nutrients and avoiding toxins. These emotions are induced by multimodal stimuli, such as taste, olfactory, and somatosensory inputs, and their dysregulation is evident in various psychiatric disorders. To understand the biological basis of liking and disgust, it is crucial to establish an animal model that allows for objective measurement of liking and disgust in response to multimodal stimuli. The only readout shared in many species including humans for liking and disgust has been the taste reactivity. However, the readouts for non-taste stimuli-induced emotions remain unestablished. Here, we show that intraoral administration of capsaicin, a chemosomatosensory stimulus, elicits orofacial and somatic reactions in male mice which are similar to those observed in taste reactivity. Capsaicin induced liking reactions at low concentrations and disgust reactions at high concentrations. Capsaicin-induced disgust reactions consisted of various reactions, including gaping and forelimb flailing, with the proportion of each reaction among disgust reactions similar to that induced by bitter and sour stimuli. These findings indicate that the orofacial and somatic reactions defined as taste reactivity are elicited not only by taste stimuli but also by intraoral chemosomatosensory stimuli, suggesting that taste reactivity can be renamed as orosensory reactivity. Understanding the biological basis of orosensory reactivity would advance our comprehension of the fundamental mechanisms underlying liking and disgust across different sensory modalities.

Keywords

Chemosomatosensory, orosensory, orofacial, taste reactivity, emotion

Introduction

The emotional responses of liking and disgust are fundamental to many animals including humans, influencing our daily life and well-being. Liking, or pleasure, is one of the primitive positive emotions elicited by the taste of sweet food or affectionate contact and is thought to have evolved to promote nutrient intake and reproduction (Berridge and Kringelbach, 2008; 2015). Disgust is one of the basic negative emotions induced by the distaste for certain foods and contact with feces and is believed to have evolved as a protective mechanism against toxins and pathogens (Darwin, 1872/1965; Rozin and Fallon, 1987; Curtis et al., 2004; Chapman and Anderson, 2012; Tybur et al., 2013).

Liking and disgust are induced by external stimuli of all five modalities in humans: taste (Steiner, 1979), olfactory (Engen and McBurney, 1964), somatosensory (Rozin and Schiller, 1980), auditory (Scott et al., 1997), and visual stimuli (Adolphs et al., 1994). The expressions of liking and disgust are dysregulated in various psychiatric disorders: anhedonia in which liking is diminished is associated with depression (Gorwood, 2008), Parkinson's disease (Loas et al., 2012), and schizophrenia (Der-Avakian and Markou, 2012; Lambert, 2018), while excessive disgust is associated with depression, anxiety, and obsessive-compulsive disorder (Davey, 2011).

To understand the biological basis of liking and disgust in health and disease, it is crucial to establish a quantitative method for estimating the degree of liking and disgust in animals. The taste reactivity test is the gold standard method to quantitatively estimate the degree of liking and disgust in animals which quantifies orofacial and somatic reactions consisting of orofacial, head, and upper-limb motor reactions induced by taste stimuli (Grill and Norgren, 1978; Berridge, 2000). The orofacial and somatic reactions defined in the taste reactivity test are highly conserved in mammals including humans, monkeys, and rodents, and are widely used as a reliable readout of liking and disgust induced by taste stimuli (Berridge, 2000). However, the application of this method has been limited to taste stimuli, raising the suspicion that this method may limit our understanding of the biological basis of liking and disgust, potentially overlooking the roles of other sensory modalities in these emotional responses.

The trigeminal sensory nerve contains C- and A-delta-fibers that convey nociceptive signals from the oral catity (Edvisson et al., 2019). C- and A-delta-fibers are excited by capsaicin, a ligand for transient receptor potential vanilloid 1 receptor (LaMotte et al., 1992; Caterina et al., 2000; Ringkamp et al., 2001). Capsaicin is widely used as a noxious

81 somatosensory stimulus in pain research (Winter et al., 1995; Ngom et al., 2001; Baad-Hansen
82 et al., 2003; O'Neill et al, 2012; Zhao et al., 2012; Lu et al., 2013; Naganawa et al., 2015). In
83 humans, intraoral administration of capsaicin can elicit both liking and disgust at varying
84 concentrations with pain induced by high concentration, although there are significant
85 individual differences (Rozin and Schiller, 1980; Rozin et al., 1982; Stevenson and Yeomans,
86 1993; 1995; Tornwall et al., 2012; Byrnes and Hayes, 2016). In rodents, high concentration of
87 capsaicin is avoided, with a preference for water over any concentrations of capsaicin
88 (Bachmanov et al, 1996; Simons et al., 2001; Furuse et al., 2002; Narukawa and Misaka, 2021).
89 However, it remains unclear whether intraoral capsaicin induces liking or disgust reactions.
90 In this study, we examined orofacial and somatic reactions representing liking and
91 disgust by three different concentrations of intraoral capsaicin in male mice and compared them
92 with those induced by two representative tastants, bitter quinine, and sour citric acid.
93

Materials and methods

Animals

The adult (6-7 weeks-old) male C57BL/6J mice were obtained from Japan SLC, Inc. (Shizuoka, Japan). Upon arrival, mice were housed in clear plastic cages (18 × 26 × 13 cm) with cellulose fiber chips (Alpha-Dri; Shepherd Specialty Papers, Kalamazoo, MI, USA) in groups of 2-4 males per cage and were then housed individually in smaller cages (14 × 21 × 12 cm) just before the beginning of handling. Mice were maintained at 22 ± 1 ˚C under 12 h light/dark cycles (lights on at 8:00 am) and given *ad libitum* access to food (CE-2; CLEA Japan Inc., Tokyo, Japan) and tap water. A tap water was changed to MilliQ water 3days before begging reactional experiments. All reactional experiments were performed in the light cycle. All the animal experiments were approved (No. A2022-005C6) by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University and performed under the relevant guidelines and regulations.

Surgery

The surgery for the implantation of an intraoral tube was performed as described previously (Tanaka et al., 2019) with minor modifications. Mice were anesthetized by intraperitoneal injection of a mixture of midazolam (5.2 mg/kg body weight (BW), Dormicum; Astellas Pharma Inc., Tokyo, Japan), butorphanol (6.5 mg/kg BW, Vetorphale; Meiji Animal Health Co., Ltd., Kumamoto, Japan), and medetomidine (0.39 mg/kg BW, Domitor; Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan). The depth of anesthesia was maintained at a sufficient level to prevent the paw pinch reflex. The eyes were protected by applying an ophthalmic ointment (Tarivid; Santen, Osaka, Japan). The epidermis of the parietal was cut in an elliptical shape and removed. A curved needle attached to an intraoral polyethylene tube (SP-10; Natsume Seisakusho Co., Ltd., Tokyo, Japan) was inserted from the incision site and advanced subcutaneously posterior to the eye to exit at a point lateral to the first maxillary molar on the right side of the mouth. The intraoral end of the tube was heat-flared to an approximate diameter of 1 mm to prevent it from being drawn into the oral mucosa. Using ear bars, the mice were mounted in a stereotaxic frame (Stoelting Co., Wood Dale, IL, USA). A piece of plastic bar was fixed to the skull with dental cement (Super-Bond C&B; Sun Medical, Shiga, Japan). The mice received subcutaneous injections of the antibiotic chloramphenicol sodium succinate (60 mg/kg BW, Chloromycetin Succinate; Daiichi Sankyo Co., Ltd., Tokyo, Japan) for infection prevention and carprofen (5 mg/kg BW, Rimadyl; Zoetis, Tokyo, Japan) for pain relief, and received intraperitoneal injection of atipamezole (0.39 mg/kg BW, Antisedan;

Noppon Zenyaku Kogyo Co., Ltd.) for reversing the anesthetic effects of medetomidine.

3 days after surgery, one end of a delivery tube (SP-10; Natsume Seisakusho Co., Ltd.) was connected to the end of the intraoral tube fixed to the plastic bar on the mouse head using a connector (KN-394, two directions (0.3+0.3); Natsume Seisakusho Co., Ltd.), and the other end was connected to a needle (30Gx13mm; ReactSystem Co., Osaka, Japan) attached to a 1-mL syringe, and ~20 μL of MilliQ water was infused into the mouth of the animal to test the patency of the tubes. The intraoral tube was flushed with ~20 μL of MilliQ water every weekday to prevent occlusion. The mice were allowed 1-2 weeks to recover from the surgery before beginning reactional experiments.

Taste reactivity tests

The taste reactivity test (Grill and Norgren, 1978; Berridge, 2000) was used to measure the affective reactional reactions of mice in response to intraoral stimulation. The test chamber consists of a glass or acrylic floor and an acrylic cylinder (30 cm height, 13 cm outside diameter, 3 mm thickness). A digital video camera (HDR-PJ800; Sony, Tokyo, Japan or HC-VX992MS; Panasonic, Osaka, Japan) was located beneath the glass floor to video record a ventral view of the mouse. On the first day of reactional experiments, the mice were put into the chamber for 15 min for habituation. From the second day, mice were put into the chamber and received intraoral infusions of 80 μL MilliQ water for 2min by a syringe pump (Legato 111; KD Scientific Inc., Holliston, MA, USA) for habituation. After that, all intraoral infusions were performed in the chamber, consisting of 80 μL solution, and lasted for 2 min by using the syringe pump. From the 9th day, mice started to receive infusions of following 4 different substances in order, each with 3 different concentrations: 0.44 mM, 4.4 mM, 44 mM tannic acid (code#32616-02; Nacalai tesque), 3.3 μM, 33 μM, and 330 μM capsaicin (code#07127-21; Nacalai tesque, Kyoto, Japan), 0.1 mM, 1 mM, 10mM quinine (code#22630; Sigma-Aldrich Co., MO, USA), and 0.01 M, 0.1 M, 1 M citric acid (code#09109-85; Nacalai tesque). Data from tannic acid stimulation were not analyzed in the present study since we noticed, after completion of the experiments, that tannic acid appears to stimulate not only the somatosensory pathway but also the taste pathway (Soares et al., 2020) and thus it was difficult to interpret that tannic acid-induced reactions are induced by somatosensory stimulation. For each substance, the test consists of two consecutive days. On Day 1, mice received an infusion of the lowest concentration of the substance. This was repeated twice. On Day 2, mice received infusions of 3 different concentrations of substance, starting from the lowest concentration to the highest concentration. The reaction of mice during infusions was video

recorded for subsequent frame-by-frame video analysis.

Taste reactivity video scoring

Manual video analysis was performed as described previously (Tanaka et al., 2019) with minor modifications. Liking reactions included midline or lateral tongue protrusion (emergence of the anterior tip of the tongue, which pushes both left and right upper lips or either left or right lip, respectively), and paw licking (licking with tongue emergence toward the mouse's forepaws, with the paws held close to the mouth). The number of tongue protrusions was quantified as the sum of the number of midline and lateral tongue protrusions. Disgust reactions included gape (large opening of the mouth with a retraction of the lower lip), headshake (rapid lateral movement of the head), face wash (wipes over the face with the paws), forelimb flail (rapid waving of both forelimbs), and floor and wall chin rub (pushing the chin against the floor or wall of the test chamber, respectively). The number of chin rubs was quantified as the sum of the number of floor and wall chin rubs. Neutral reactions (less strongly liked to liking/disgust evaluations) were considered to be rhythmic mouth movements (mouth movements without the emergence of the tongue) and ordinary grooming (Sequential movements of behavior that include movements that bring the mouth close to the abdomen or genital area). To be sure that each component of taste reactivity contributes equally to the final scores, reactions that occurred in continuous bouts were scored in time bins (Berridge, 2000) with minor modifications. Components characterized by long-duration bouts, such as paw licking, face wash, mouth movements, and grooming, were scored in five-second bins (successive repetitions within five seconds were scored as one occurrence). Components characterized by moderate-duration bouts, such as chin rub were scored in two-second bins. The other reactions, such as tongue protrusion and forelimb flail that can occur as a single reaction were scored as separate occurrences (e.g., one forelimb flail equals one occurrence). A liking reaction total was quantified as the sum of scores for tongue protrusions and paw licking. A disgust reaction total was quantified as the sum of gape, headshake, face wash, forelimb flail, and chin rub scores. A neutral reaction total was quantified as the sum of mouth movements and grooming.

Statistical analysis

Differences between the three groups were analyzed by one-way ANOVA followed by Tukey's multiple-comparison (Tukey's) test. The association between the magnitude of capsaicin-induced disgust reactions and the logarithm of the concentration of the solution was analyzed

using Pearson correlation coefficient. A p-value < 0.05 was considered statistically significant. All tests were two-tailed. Most data were expressed as line graphs with dots representing the group mean and error bars representing the standard error of the mean (SEM) or truncated violin plots with gray bars representing the group median and dotted lines representing the interquartile range. Numerical data represented in the main text is the mean ± SEM.

Results

To investigate orofacial and somatic reactions induced by intraoral infusion of capsaicin and compare them with those for quinine and citric acid, three different concentration solutions for each stimulus were infused. The sum of scores of tongue protrusion and paw licking was assessed as the liking reaction total (Berridge, 2000). The sum of scores of gape, headshake, face wash, forelimb flail, and chin rub was assessed as the disgust reaction total. The low (3.3 μM)- and middle (33 μM)-concentration capsaicin induced liking reactions (6.7 ± 1.8 scores/ minute at 3.3 μM; 5.8 ± 1.7 at 33 μM; Fig. 1), which are comparable to those induced by the sweet saccharin solution in our previous study (6.9 ± 1.6; Tanaka et al., 2019). A weaker but similar elicit of liking reactions was observed in citric acid (Fig. 1B, C) consistent with previous studies (Brining et al., 1991; Galaverna et al., 1993; Steiner, 2001). Any concentration of quinine induced almost no liking reactions (Fig. 1B, C). Capsaicin-induced liking reactions were not almost completely induced at the highest concentration (0.1 ± 0.1 scores/ minute; Fig. 1A, B). At the lowest concentrations for each stimulus, all of three stimuli induced few disgust reactions (1.9 ± 0.7 at 3.3 μM capsaicin, 4.8 ± 1.9 at 10^2 μM quinine, 2.9 ± 0.6 at 10^4 μM citric acid; Fig. 2), which are comparable to those induced by the water infusion in our previous study (2.4 ± 1.0; Tanaka et al., 2019). At the highest concentrations, all of three stimuli induced significantly higher scores of disgust reactions compared to those at lower concentrations (Fig. 2; sFig.1). Thus, capsaicin elicits both liking reactions and disgust reactions depending on its concentration.

In the following, we further analyzed capsaicin-induced liking reactions and disgust reactions to characterize them in comparison with those induced by quinine and citric acid. Previous studies have shown that the magnitude of sensation is proportional to the logarithm of the magnitude of the stimulus (Fechner, 1860/1966). We found that the magnitude of capsaicin-induced disgust reactions was proportional to the logarithm of the magnitude of the stimulus, i.e. the logarithm of the concentration of the solution (Fig. 2B). This logarithmic association was notably pronounced for capsaicin (Pearson's $r = 0.998$, $P = 0.031$), as oppose to quinine ($r = 0.934$, $P = 0.232$) and citric acid ($r = 0.927$, $P = 0.246$; Fig. 2B). The proportionality of the magnitude of capsaicin-induced liking reactions to the logarithm of the concentration of the solution was not able to be analyzed, since their scores reached at maximum value even at the lowest concentration (3.3 μM) of capsaicin in the present study (Fig. 1B, C). These findings suggest that capsaicin-induced disgust reactions at least, may reflect capsaicin-induced sensations such as burning pain, hotness, and disgust (Rozin and Schiller, 1980; Rozin et al., 1982; Stevenson and Yeomans, 1993; 1995; Tornwall et al., 2012;

236 Byrnes and Hayes, 2016).

237 As the concentration of capsaicin increases, the disgust reactions increase, and the
238 liking reactions decrease as a whole (Fig.3A). A similar tendency was observed in citric acid
239 (Fig.3A). The mechanistic relationship between the liking reactions and the disgust reactions
240 could potentially be one of the following two (Fig. 3B). First, the liking reactions decrease as
241 the disgust reactions increase in a seesaw manner. In this *seesaw* model, the increase of disgust
242 reactions always accompanies with the decrease of liking reactions. Second, the liking
243 reactions change independently from the disgust reactions. In this *independent* model, when
244 disgust reactions increase, liking reactions may increase as well. We analyzed how disgust
245 reactions and liking reactions of each mouse changed as the concentration of capsaicin or citric
246 acid solution increased from low concentrations to medium concentrations and from medium
247 concentrations to high concentrations. For the change from low concentrations to medium
248 concentrations of capsaicin (Fig. 3C) and citric acid (Fig. 3D) solutions, all mice showed an
249 increase in disgust reactions, and approximately half of them also showed an increase in liking
250 reactions, in consistent with an *independent* model. For the change from medium
251 concentrations to high concentrations of capsaicin (Fig. 3E) and citric acid (Fig. 3F) solutions,
252 most mice showed an increase in disgust reactions, and most of them showed a decrease in
253 liking reactions, in consistent with a *seesaw* model. These data suggest that mechanistic
254 relationship between liking reactions and disgust reactions is dynamically regulated depending
255 on the intensity of the chemosomatosensory and taste stimuli that induce them.

256 The previous studies suggest that there are two characteristics that must be met for
257 liking reaction and disgust reaction to be considered as indicators of liking and disgust in
258 animals (Berridge, 2000). The first is that the multiple orofacial and somatic reactions that
259 comprise each of the liking reaction and disgust reaction should show a similar direction of
260 change (increase or decrease) (Fig. 4A, B). Even if the liking reaction or disgust reaction
261 changes computationally as a result of changes in a single orofacial and somatic reaction, such
262 change of the liking reaction or disgust reaction may be doubtful as an indicator of changes in
263 liking or disgust. To investigate whether capsaicin-induced liking reactions and disgust
264 reactions met the this first characteristic as well as taste-induced ones, we analyzed each
265 reaction consisting of liking reactions and disgust reactions. As for liking reactions defined as
266 the sum of scores of tongue protrusion and paw licking, only tongue protrusion was observed
267 (Fig.4C, Supplemental fig. 2A) and paw licking was never observed in all tests in all mice. On
268 the other hand, the middle- and high-concentration capsaicin increased various disgust
269 reactions as well as high-concentration quinine and citric acid in a concentration-dependent

manner (Fig. 4D, Supplemental fig. 2B), although head shake was rarely observed in all tested conditions. Thus, disgust reactions induced by middle- and high-concentration capsaicin can pass the first criteria as well as those induced by high-concentration quinine and citric acid. Second, when the liking reaction or disgust reaction changes, another reaction and neutral reaction remain unchanged or change in the opposite direction (Fig. 5A, B). As for liking reactions, the low- and middle-concentration capsaicin increased the liking reactions compared to high-concentration capsaicin, and simultaneously, decreased disgust reactions (Fig. 5C). However, the low- and middle-concentration capsaicin increased neutral reactions (Fig. 5C, Supplemental fig. 3A, B). On the other hand, the high-concentration capsaicin increased the disgust reactions, and simultaneously, decreased both liking reactions and neutral reactions (Fig. 5C, Supplemental fig. 3A, B). The middle-concentration capsaicin increased the disgust reactions, and did not significantly change both liking reactions and neutral reactions (Fig. 5C, Supplemental fig. 3A, B). The high-concentration quinine (Fig. 5D) and citric acid (Fig. 5E) induced largely similar reactions with middle- and high-concentration capsaicin (Fig. 5C). As for each reaction categorized in neutral reactions, mouth movements were significantly decreased by high-concentration capsaicin compared to low- and middle-concentration capsaicin (Supplemental fig. 3C, D), while grooming was never observed in all tests in all mice. Thus, disgust reactions induced by middle- and high-concentration capsaicin can pass the second criteria as well as those induced by high-concentration quinine and citric acid. Taken together, while liking reactions induced by low- and middle-concentration capsaicin pass neither the first nor second criteria, disgust reactions induced by middle- and high-concentration capsaicin can pass both the first and second criteria as well as those induced by high-concentration quinine and citric acid.

Previous studies suggest that as the total disgust score was increased, the ratio of each disgust reaction among all disgust reactions change: gape appeared first at lowest disgust score in total, then chin rub was emitted as total disgust score increase, and finally forelimb flail appeared (Breslin et al., 1992). To determine if similar changes in reaction are observed in capsaicin stimulation, we analyzed the ratios of each disgust reaction among all disgust reactions at different concentration of capsaicin and compared them with those induced by quinine and citric acid. As the concentration of solution is increased, ratio of each disgust reaction change similarly among capsaicin, quinine, and citric acid: gape and headshake were observed form the beginning and tended to be decreased as the concentration of solution is increased (Fig. 6A, C), face wash and forelimb flail were constantly observed in all concentrations (Fig. 6D, E), and the ratio of chin rub was significantly increased at high

304 concentration (Fig. 6B, F, Supplemental fig. 4). These data suggest that the components of
305 disgust reactions dynamically regulated depending on the intensity of the chemosomatosensory
306 and taste stimuli that induce them.
307

Discussion

The present study demonstrates that intraoral capsaicin stimulation elicits liking reactions at low concentrations (Fig. 1) and disgust reactions at a high concentration (Fig. 2) in mice. This is the first demonstration that non-taste stimuli elicited liking and disgust reactions defined as taste reactivity (Grill and Norgren, 1978; Berridge, 2000). Given that liking and disgust reactions may ordinally underlie subjective liking and disgust (Berridge, 2000), the present results pave the way for the liking and disgust reactions to be used as objective readouts of subjective liking and disgust irrespective of stimulation modalities in mice.

The taste reactivity would be better renamed the orosensory reactivity

The present study demonstrates that chemosomatosensory stimuli, capsaicin elicits both liking reactions (Fig. 1) and disgust reactions (Fig. 2) defined as taste reactivity. The taste reactivity has been established as a gold standard method for estimating liking and disgust in human neonates and animals by quantifying the orofacial and somatic reactions consisting of orofacial, head, and upper limb motor reactions induced by intraoral taste stimuli (Grill and Norgren, 1978; Berridge, 2000). The present study demonstrates that chemosomatosensory stimuli also induce similar orofacial and somatic reactions, prompting us to propose that the taste reactivity is renamed the orosensory reactivity, since the description, for example, that the taste reactivity induced by non-taste stimuli (e.g. capsaicin) would be confusing and misleading. In addition, this rename would appropriately move our understanding of the gold standard method from a taste-limited method to a multimodal one.

Trigeminal somatosensory information is sufficient to induce liking reactions

The present study shows that intraoral capsaicin stimulation elicits liking reactions at a low concentration (Fig. 1). Given that transection of the trigeminal sensory nerve reduces liking reaction to taste stimuli (Berridge and Fentress, 1985), trigeminal somatosensory information is required (Berridge and Fentress, 1985) and sufficient (Fig. 1) to induce liking reactions against an intraoral solution. It remains unclear how the transection of the trigeminal sensory nerve reduces taste-induced liking reactions, and what the difference is between taste-induced liking reactions and somatosensory-induced ones in terms of their underlying neural mechanisms.

Mice may like low-concentration capsaicin as well as humans

Capsaicin is the principal irritant in the chili pepper, probably the most widely consumed spice

342 in the world (Rozin and Schiller, 1980). The intraoral 3-13.2 μM capsaicin induces liking in
343 humans with large individual differences (Rozin and Schiller, 1980; Rozin et al., 1982;
344 Stevenson and Yeomans, 1993; 1995; Tornwall et al., 2012; Byrnes and Hayes, 2016). It has
345 been believed that only humans like capsaicin, and other animals do not (Rozin and Schiller,
346 1980). Indeed, C57BL/6 mice do not prefer any concentration of capsaicin solution (0.1-99
347 μM) to water and even avoid more than 1 μM capsaicin solution in the two-bottle preference
348 test (Bachmanov et al, 1996; Simons et al., 2001; Furuse et al., 2002; Narukawa and Misaka,
349 2021). The present study demonstrates that intraoral low-concentration (3.3 μM) capsaicin
350 induces liking reactions but not disgust reactions in C57BL/6 mice (Fig. 1, 2, 3A). Thus,
351 C57BL/6 mice show sole liking reactions to 3.3 μM capsaicin solutions in the orosensory
352 reactivity test but are likely to avoid them in the two-bottle preference test (Bachmanov et al,
353 1996; Simons et al., 2001; Furuse et al., 2002; Narukawa and Misaka, 2021). These
354 seemingly conflicting results are interesting but not surprising since it is well known that the
355 liking reactions in taste reactivity reflect a hedonic core process (Berridge, 2000) but not intake
356 motivation measured in the choice tests (Bachmanov et al, 1996; Simons et al., 2001; Furuse
357 et al., 2002; Narukawa and Misaka, 2021).
358 Both capsaicin-induced liking reactions and citric acid-induced liking reactions were
359 completely dominated by tongue protrusions (compare Fig. 1C with Fig. 4C). Chemical
360 irritants such as formalin, mustard oil and capsaicin injected into the paw often induce licking,
361 biting, and shaking of the paw in rodents (Koepp et al., 2006; Kwan et al., 2006; Sakurada et
362 al., 2009). One may argue that capsaicin-induced tongue protrusions reflect licking behavior
363 against chemical nociception. Of note, the highest-concentration (330 μM) capsaicin, of
364 which concentration is much higher than that induced intraoral pain in humans (30 μM) (Ngom
365 et al., 2001), did not induce tongue protrusion (Fig. 4C) but induced disgust (Fig.2C),
366 suggesting that potentially painful capsaicin stimulus in the oral cavity does not necessarily
367 induce tongue protrusion. Thus, the tongue protrusions induced by intraoral low-
368 concentration capsaicin likely represent liking but not licking against chemical nociception.
369 Taken together, the present results support the notion that mice like 3.3 μM capsaicin as well
370 as humans. To complement the evolutionary gap between liking reactions in mice in the
371 present study and conscious liking ratings in humans (Rozin and Schiller, 1980; Rozin et al.,
372 1982; Stevenson and Yeomans, 1993; 1995; Tornwall et al., 2012; Byrnes and Hayes, 2016), it
373 would be interesting to test whether not only sweet taste (Steiner, 2001) but also 3.3 μM
374 capsaicin induces liking reactions in human neonates or monkeys.
375

Mice may experience disgust from intraoral middle- and high-concentration capsaicin

While 3.3 μM capsaicin infusion induced few disgust reactions (Fig. 2B, C) comparable to water infusion (2.4 ± 1.0; Tanaka et al., 2019), 33 μM and 330 μM capsaicin infusion induced significant disgust reactions (Fig. 2 B, C). 33 μM capsaicin also induced significant liking reactions, suggesting that it induced both liking and disgust according to the two-dimensional account of palatability (Berridge and Grill, 1983; 1984). Given that 33 μM and 330 μM capsaicin are higher than 12-13.2 μM capsaicin that induced unpleasantness or disliking in humans with large individual differences (Stevenson and Yeomans, 1993; Byrnes and Hayes, 2016), 33 μM and 330 μM capsaicin infusion may induce not only disgust reactions but also unpleasantness, disliking, and/or disgust in mice. To assess this hypothesis, it would be crucial to test whether intraoral 33 μM or 330 μM capsaicin induces disgust in humans. In addition, to complement the evolutionary gap between disgust reactions in mice and conscious disgust ratings in humans, it would be interesting to test whether not only bitter taste (Steiner et al., 2001) but also 33 μM or 330 μM capsaicin induces disgust reactions in human neonates or monkeys.

Intraoral middle- and high-concentration capsaicin may induce not only disgust reactions but also pain in mice

Again, in the present study, intraoral 33 μM and 330 μM capsaicin induced disgust reactions (Fig. 2B, C). Given that 33 μM and 330 μM capsaicin are in the range of 30 μM-165 mM capsaicin that induces intraoral pain in humans (Ngom et al., 2001; Baad-Hansen et al., 2003; Zhao et al., 2012; Lu et al., 2013; Naganawa et al., 2015), intraoral 33 μM and 330 μM capsaicin are likely to induce not only disgust reactions but also pain in mice. Although the relationship between disgust and pain remains unclear, they can be tightly linked: disgust and pain share some facial action units (Kappesser and Williams, 2002; Kunz et al., 2013) and neuronal responses in humans (Corradi-Dell'Acqua et al., 2016). It would be interesting to investigate the similarities and differences between disgust and pain in terms of underlying neural mechanisms.

Capsaicin-induced disgust reactions to investigate the mechanism of oral disorders in mice

Assessing disgust and pain levels arising from the oral cavity in an awake animal model is essential for elucidating the mechanism for the pathophysiology of oral discomfort and pain syndromes including oral cenesthopathy (Umezaki et al., 2016) and burning mouth syndrome

410 (Klein et al., 2020). However, no awake-animal model is available so far, probably because
411 it is believed that experimental manipulation of intraoral tissues is challenging in awake
412 animals (Terayama et al., 2014). The present study utilized a taste reactivity procedure
413 developed in the research field of taste and emotion (Grill and Norgren, 1978; Berridge, 2000)
414 to stimulate intraoral tissue with high-concentration capsaicin that induces unpleasantness
415 (Stevenson and Yeomans, 1993; Byrnes and Hayes, 2016) and pain (Ngom et al., 2001; Baad-
416 Hansen et al., 2003; Zhao et al., 2012; Lu et al., 2013; Naganawa et al., 2015) in humans and
417 detected disgust reactions induced by the stimulation (Fig. 2B, C). Thus, capsaicin-induced
418 disgust reactions may be able to be used as a readout of intraoral discomfort and pain in mice
419 for elucidating the mechanism for the pathophysiology of oral discomfort and pain syndromes
420 (Umezaki et al., 2016; Klein et al., 2020). As the first step to assess this assumption, it would
421 be interesting to test whether effective drugs for oral cenesthopathy such as aripiprazole
422 (Umezaki et al., 2018), and for burning mouth syndrome such as clonazepam (Klein et al.,
423 2020) suppress capsaicin-induced disgust reactions.
424

Conflict of interests

The authors declare no competing interests.

Funding

This work was supported by JSPS KAKENHI Grant Numbers JP19K06938 and TMDU priority research areas grant to D.H.T.

Acknowledgments

We thank Mr. Shizuki Inaba and other lab members for their helpful comments and discussions.

References

Adolphs, R., Tranel, D., Damasio, H. & Damasio, A. (1994) Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. Nature, 372, 669-672.

Baad-Hansen, L., Jensen, T.S. & Svensson, P. (2003) A human model of intraoral pain and heat hyperalgesia. J Orofac Pain, 17, 333-340.

Bachmanov, A.A., Tordoff, M.G. & Beauchamp, G.K. (1996) Ethanol consumption and taste preferences in C57BL/6ByJ and 129/J mice. Alcohol Clin Exp Res, 20, 201-206.

Berridge, K.C. (2000) Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. Neurosci Biobehav Rev, 24, 173-198.

Berridge, K.C. & Fentress, J.C. (1985) Trigeminal-taste interaction in palatability processing. Science, 228, 747-750.

Berridge, K.C. & Grill, H.J. (1983) Alternating ingestive and aversive consummatory responses suggest a two-dimensional analysis of palatability in rats. Behav Neurosci, 97, 563-573.

Berridge, K.C. & Grill, H.J. (1984) Isohedonic tastes support a two-dimensional hypothesis of palatability. Appetite, 5, 221-231.

Berridge, K.C. & Kringelbach, M.L. (2008) Affective neuroscience of pleasure: reward in humans and animals. Psychopharmacology (Berl), 199, 457-480.

Berridge, K.C. & Kringelbach, M.L. (2015) Pleasure systems in the brain. Neuron, 86, 646-664.

Breslin, P.A., Spector, A.C. & Grill, H.J. (1992) A quantitative comparison of taste reactivity behaviors to sucrose before and after lithium chloride pairings: a unidimensional account of palatability. Behav Neurosci, 106, 820-836.

Brining, S.K., Belecky, T.L. & Smith, D.V. (1991) Taste reactivity in the hamster. Physiol Behav, 49, 1265-1272.

Byrnes, N.K. & Hayes, J.E. (2016) Behavioral measures of risk tasking, sensation seeking and sensitivity to reward may reflect different motivations for spicy food liking and consumption. Appetite, 103, 411-422.

Caterina, M.J., Leffler, A., Malmberg, A.B., Martin, W.J., Trafton, J., Petersen-Zeitz, K.R., Koltzenburg, M., Basbaum, A.I. & Julius, D. (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science, 288, 306-313.

Chapman, H.A. & Anderson, A.K. (2012) Understanding disgust. Ann N Y Acad Sci, 1251, 62-76.

Corradi-Dell'Acqua, C., Tusche, A., Vuilleumier, P. & Singer, T. (2016) Cross-modal representations of first-hand and vicarious pain, disgust and fairness in insular and

cingulate cortex. Nat Commun, 7, 10904.

Curtis, V., Aunger, R. & Rabie, T. (2004) Evidence that disgust evolved to protect from risk of disease. Proc Biol Sci, 271 Suppl 4, S131-133.

Darwin, C. (1965) The expression of the emotions in man and animals. Chicago: University of Chicago Press (Original work published 1872).

Davey, G.C. (2011) Disgust: the disease-avoidance emotion and its dysfunctions. Philos Trans R Soc Lond B Biol Sci, 366, 3453-3465.

Der-Avakian, A. & Markou, A. (2012) The neurobiology of anhedonia and other reward-related deficits. Trends Neurosci, 35, 68-77.

Edvinsson, J.C.A., Warfvinge, K., Krause, D.N., Blixt, F.W., Sheykhzade, M., Edvinsson, L. & Haanes, K.A. (2019) C-fibers may modulate adjacent Adelta-fibers through axon-axon CGRP signaling at nodes of Ranvier in the trigeminal system. J Headache Pain, 20, 105.

Engen, T. & McBurney, D.H. (1964) Magnitude and Category Scales of the Pleasantness of Odors. J Exp Psychol, 68, 435-440.

Fechner. G.T. (1966). Elements of psychophysics (Vol. I) (E.G. Boring & D.H. Howes, Eds.; H.E. Adler, Trans.). New York: Holt. Rinehart & Winston. (Original work published 1860)

Feurte, S., Nicolaidis, S. & Berridge, K.C. (2000) Conditioned taste aversion in rats for a threonine-deficient diet: demonstration by the taste reactivity test. Physiol Behav, 68, 423-429.

Galaverna, O.G., Seeley, R.J., Berridge, K.C., Grill, H.J., Epstein, A.N. & Schulkin, J. (1993) Lesions of the central nucleus of the amygdala. I: Effects on taste reactivity, taste aversion learning and sodium appetite. Behav Brain Res, 59, 11-17.

Gorwood, P. (2008) Neurobiological mechanisms of anhedonia. Dialogues Clin Neurosci, 10, 291-299.

Grill, H.J. & Norgren, R. (1978) The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res, 143, 263-279.

Kappesser, J. & Williams, A.C. (2002) Pain and negative emotions in the face: judgements by health care professionals. Pain, 99, 197-206.

Klein, B., Thoppay, J.R., De Rossi, S.S. & Ciarrocca, K. (2020) Burning Mouth Syndrome. Dermatol Clin, 38, 477-483.

Koepp, J., Lindsey, C.J., Motta, E.M. & Rae, G.A. (2006) Role of the paratrigeminal nucleus in nocifensive responses of rats to chemical, thermal and mechanical stimuli applied to the hind paw. Pain, 122, 235-244.

Kunz, M., Peter, J., Huster, S. & Lautenbacher, S. (2013) Pain and disgust: the facial signaling of two aversive bodily experiences. PLoS One, 8, e83277.

Kwan, K.Y., Allchorne, A.J., Vollrath, M.A., Christensen, A.P., Zhang, D.S., Woolf, C.J. & Corey, D.P. (2006) TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron, 50, 277-289.

Lambert, C., Da Silva, S., Ceniti, A.K., Rizvi, S.J., Foussias, G. & Kennedy, S.H. (2018) Anhedonia in depression and schizophrenia: A transdiagnostic challenge. CNS Neurosci Ther, 24, 615-623.

LaMotte, R.H., Lundberg, L.E. & Torebjork, H.E. (1992) Pain, hyperalgesia and activity in nociceptive C units in humans after intradermal injection of capsaicin. J Physiol, 448, 749-764.

Loas, G., Krystkowiak, P. & Godefroy, O. (2012) Anhedonia in Parkinson's disease: an overview. J Neuropsychiatry Clin Neurosci, 24, 444-451.

Lu, S., Baad-Hansen, L., List, T., Zhang, Z. & Svensson, P. (2013) Somatosensory profiling of intra-oral capsaicin and menthol in healthy subjects. Eur J Oral Sci, 121, 29-35.

Naganawa, T., Baad-Hansen, L., Ando, T. & Svensson, P. (2015) Influence of topical application of capsaicin, menthol and local anesthetics on intraoral somatosensory sensitivity in healthy subjects: temporal and spatial aspects. Exp Brain Res, 233, 1189-1199.

Narukawa, M. & Misaka, T. (2021) Change in Taste Preference to Capsaicin and Catechin Due to Aging in Mice. J Nutr Sci Vitaminol (Tokyo), 67, 196-200.

Ngom, P.I., Dubray, C., Woda, A. & Dallel, R. (2001) A human oral capsaicin pain model to assess topical anesthetic-analgesic drugs. Neurosci Lett, 316, 149-152.

O'Neill, J., Brock, C., Olesen, A.E., Andresen, T., Nilsson, M. & Dickenson, A.H. (2012) Unravelling the mystery of capsaicin: a tool to understand and treat pain. Pharmacol Rev, 64, 939-971.

Ringkamp, M., Peng, Y.B., Wu, G., Hartke, T.V., Campbell, J.N. & Meyer, R.A. (2001) Capsaicin responses in heat-sensitive and heat-insensitive A-fiber nociceptors. J Neurosci, 21, 4460-4468.

Rozin, P., Ebert, L. & Schull, J. (1982) Some like it hot: a temporal analysis of hedonic responses to chili pepper. Appetite, 3, 13-22.

Rozin, P. & Fallon, A.E. (1987) A perspective on disgust. Psychol Rev, 94, 23-41.

Rozin, P. & Schiller, D. (1980) The Nature and Acquisition of a Preference for Chili Pepper by Humans. Motivation and Emotion, 4, 77-101.

Sakurada, T., Kuwahata, H., Katsuyama, S., Komatsu, T., Morrone, L.A., Corasaniti, M.T., Bagetta, G. & Sakurada, S. (2009) Intraplantar injection of bergamot essential oil into the mouse hindpaw: effects on capsaicin-induced nociceptive behaviors. Int Rev Neurobiol, 85, 237-248.

Scott, S.K., Young, A.W., Calder, A.J., Hellawell, D.J., Aggleton, J.P. & Johnson, M. (1997) Impaired auditory recognition of fear and anger following bilateral amygdala lesions. Nature, 385, 254-257.

Simons, C.T., Dessirier, J.M., Jinks, S.L. & Carstens, E. (2001) An animal model to assess aversion to intra-oral capsaicin: increased threshold in mice lacking substance p. Chem Senses, 26, 491-497.

Soares, S., Brandao, E., Guerreiro, C., Soares, S., Mateus, N. & de Freitas, V. (2020) Tannins in Food: Insights into the Molecular Perception of Astringency and Bitter Taste. Molecules, 25.

Steiner, J.E. (1979) Human facial expressions in response to taste and smell stimulation. Adv Child Dev Behav, 13, 257-295.

Steiner, J.E., Glaser, D., Hawilo, M.E. & Berridge, K.C. (2001) Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates. Neurosci Biobehav Rev, 25, 53-74.

Stevenson, R.J. & Yeomans, M.R. (1993) Differences in ratings of intensity and pleasantness for the capsaicin burn between chili likers and non-Iikers; implications for liking development. Chemical Senses, 18, 471-482.

Stevenson, R.J. & Yeomans, M.R. (1995) Does exposure enhance liking for the chilli burn? Appetite, 24, 107-120.

Tanaka, D.H., Li, S., Mukae, S. & Tanabe, T. (2019) Genetic Access to Gustatory Disgust-Associated Neurons in the Interstitial Nucleus of the Posterior Limb of the Anterior Commissure in Male Mice. Neuroscience, 413, 45-63.

Terayama, R., Maruhama, K., Tsuchiya, H., Mizutani, M., Iida, S. & Sugimoto, T. (2014) Assessment of intraoral mucosal pain induced by the application of capsaicin. Arch Oral Biol, 59, 1334-1341.

Tornwall, O., Silventoinen, K., Kaprio, J. & Tuorila, H. (2012) Why do some like it hot? Genetic and environmental contributions to the pleasantness of oral pungency. Physiol Behav, 107, 381-389.

Tybur, J.M., Lieberman, D., Kurzban, R. & DeScioli, P. (2013) Disgust: evolved function and structure. Psychol Rev, 120, 65-84.

Umezaki, Y., Miura, A., Shinohara, Y., Mikuzuki, L., Sugawara, S., Kawasaki, K., Tu, T.T., Watanabe, T., Suga, T., Watanabe, M., Takenoshita, M., Yoshikawa, T., Uezato, A., Nishikawa, T., Hoshiko, K., Naito, T., Motomura, H. & Toyofuku, A. (2018) Clinical characteristics and course of oral somatic delusions: a retrospective chart review of 606 cases in 5 years. Neuropsychiatr Dis Treat, 14, 2057-2065.

Umezaki, Y., Miura, A., Watanabe, M., Takenoshita, M., Uezato, A., Toriihara, A., Nishikawa, T. & Toyofuku, A. (2016) Oral cenesthopathy. Biopsychosoc Med, 10, 20.

Winter, J., Bevan, S. & Campbell, E.A. (1995) Capsaicin and pain mechanisms. Br J Anaesth, 75, 157-168.

Zhao, N.N., Whittle, T., Murray, G.M. & Peck, C.C. (2012) The effects of capsaicin-induced intraoral mucosal pain on jaw movements in humans. J Orofac Pain, 26, 277-287.

Figure legends

Figure 1

Capsaicin of low and middle concentrations elicits liking reactions

(A) Representative time-lapse sequences of tongue protrusion during intraoral infusion of capsaicin. The numbers at the bottom of each panel indicate elapsed time (milliseconds). Scale bar, 5 mm.

(B) Relationship between the score of liking reactions during intraoral infusion of capsaicin (red circles; $n = 8$ mice from 6 independent experiments), quinine (green rectangles; $n = 6$ mice from 4 independent experiments), and citric acid (blue triangles; $n = 6$ mice from 4 independent experiments) and their concentrations. Error bars are SEM.

(C) The score of liking reactions during intraoral infusion of three different concentrations of capsaicin (left panel; $n = 8$ mice from 6 independent experiments), quinine (middle panel; $n = 6$ mice from 4 independent experiments), and citric acid (right panel; $n = 6$ mice from 4 independent experiments).

*$P < .05$, Tukey's test.

Figure 2

Capsaicin of increasing concentration elicits more disgust reactions

(A) Representative time-lapse sequences of disgust reactions during intraoral infusion of capsaicin. The numbers at the bottom of each panel indicate elapsed time (milliseconds). G, gape; HS, head shake; FW, face wash; CR, chin rub; FF, forelimb flail. Scale bar, 5 mm.

(B) Relationship between the score of disgust reactions during intraoral infusion of capsaicin (red circles; $n = 8$ mice from 6 independent experiments), quinine (green rectangles; $n = 6$ mice from 4 independent experiments), and citric acid (blue triangles; $n = 6$ mice from 4 independent experiments) and their concentrations. Error bars are SEM.

(C) The score of disgust reactions during intraoral infusion of three different concentrations of capsaicin (left panel; $n = 8$ mice from 6 independent experiments), quinine (middle panel; $n = 6$ mice from 4 independent experiments), and citric acid (right panel; $n = 6$ mice from 4 independent experiments).

*$P < .05$, ***$P < .001$, ****$P < .0001$, Tukey's test.

Figure 3

The mechanistic relationship between liking reactions and disgust reactions is dynamically regulated depending on the solution concentration

(A) Relationship between the score of liking reactions (red) and disgust reactions (black) during intraoral infusion of capsaicin (circles) and citric acid (triangles) and their concentrations. Graphs showing excerpts of the data shown in Fig. 1B and Fig. 2B overlaid on top of each other.

(B) Diagram of possible models explaining the mechanistic relationship between the liking reactions and the disgust reactions. In *seesaw* model, the increase of disgust reactions always accompanies with the decrease of liking reactions. In *independent* model, when disgust reactions increase, liking reactions may increase as well.

(C, E) Differences of the scores of liking reactions and disgust reactions by middle- (33 μM) (**C**) or high-concentration (330 μM) (**E**) capsaicin from those by low- (3.3 μM) (**C**) or middle-concentration (33 μM) (**E**) capsaicin in each mouse, respectively.

(D, F) Differences of the scores of liking reactions and disgust reactions by middle- (0.1 M) (**D**) or high-concentration (1 M) (**F**) citric acid from those by low- (0.01 M) (**D**) or middle-concentration (0.1 M) (**F**) citric acid in each mouse, respectively.

r, Pearson's *r*.

Figure 4

Various reactions consisting capsaicin-induced disgust reactions show a similar direction of change as the increase of capsaicin concentration

(A, B) Diagrams explaining that multiple orofacial and somatic reactions that comprise each of the liking reaction (**A**) and disgust reaction (**B**) should show a similar direction of change. TP, tongue protrusion; PL, paw licking; G, gape; CR, chin rub; HS, head shake; FW, face wash; FF, forelimb flail.

(C,D) The score of tongue protrusion (**C**), gape, chin rub, head shake, face wash, and forelimb flail (**D**) during intraoral infusion of three different concentrations of capsaicin (n = 8 mice from 6 independent experiments), quinine (n = 6 mice from 4 independent experiments), and citric acid (n = 6 mice from 4 independent experiments).

*$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$, Tukey's test.

Figure 5

Increase of capsaicin-induced disgust reactions accompanies with decrease or constant of liking reactions and neutral reactions

(A, B) Diagrams explaining that when the liking reaction (**A**) or disgust reaction (**B**) increase, another reaction and neutral reaction should remain unchanged or change in the opposite

direction.

(C-E) Relationship between the score of liking reactions (red), neutral reaction (green), and disgust reactions (black) during intraoral infusion of capsaicin (circles), quinine (rectancle) and citric acid (triangles) and their concentrations. Graphs showing excerpts of the capsaicin (**C**), quinine (**D**), and citric acid (**E**) data shown in Fig. 1B, Fig. 2B, and Supplemental fig. 3A overlaid on top of each other.

Figure 6

Capsaicin induces various disgust reactions in a concentration-dependent manner

(A-E) The ratio of gape (**A**), chin rub (**B**), head shake (**C**), face wash (**D**), and forelimb flail (**E**) among all disgust reactions induced by capsaicin (n = 8 mice from 6 independent experiments), quinine (n = 6 mice from 4 independent experiments), and citric acid (n = 6 mice from 4 independent experiments).

(**F**) Illustration summarizing the results in **A-E**. Gape and headshake were observed form the beginning and tended to be decreased as the concentration of solution is increased, face wash and forelimb flail were constantly observed in all concentrations, and the chin rub was increased at high concentration of capsaicin, quinine, and citric acid.

Error bars are SEM. G, gape; CR, chin rub; HS, head shake; FW, face wash; FF, forelimb flail.

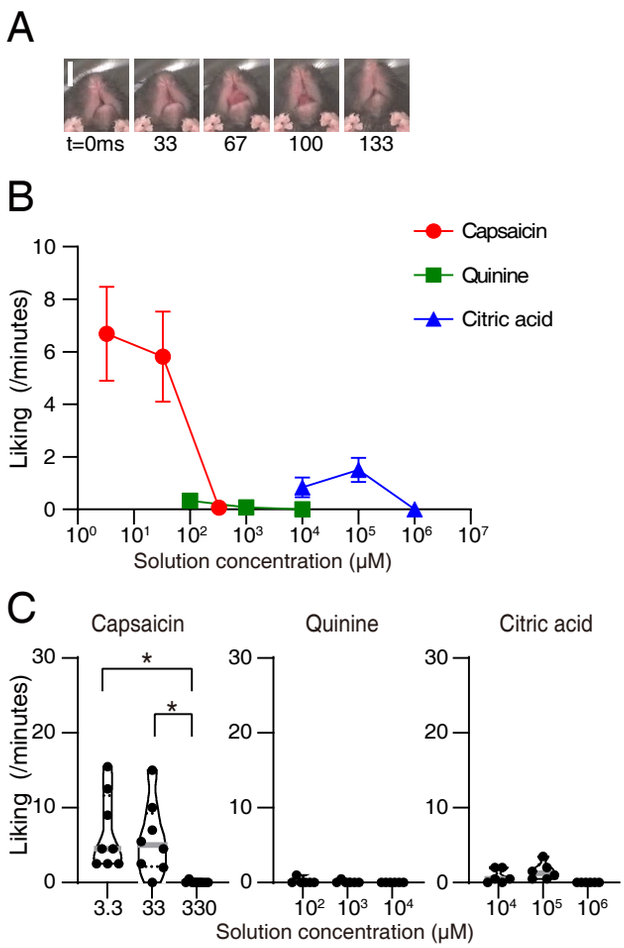
A
t=0ms
33
67
100
133
B
Capsaicin
Quinine
Citric acid
Liking (/minutes)
Solution concentration (μM)
C
Capsaicin
Quinine
Citric acid
Liking (/minutes)
Solution concentration (μM)

Figure 1

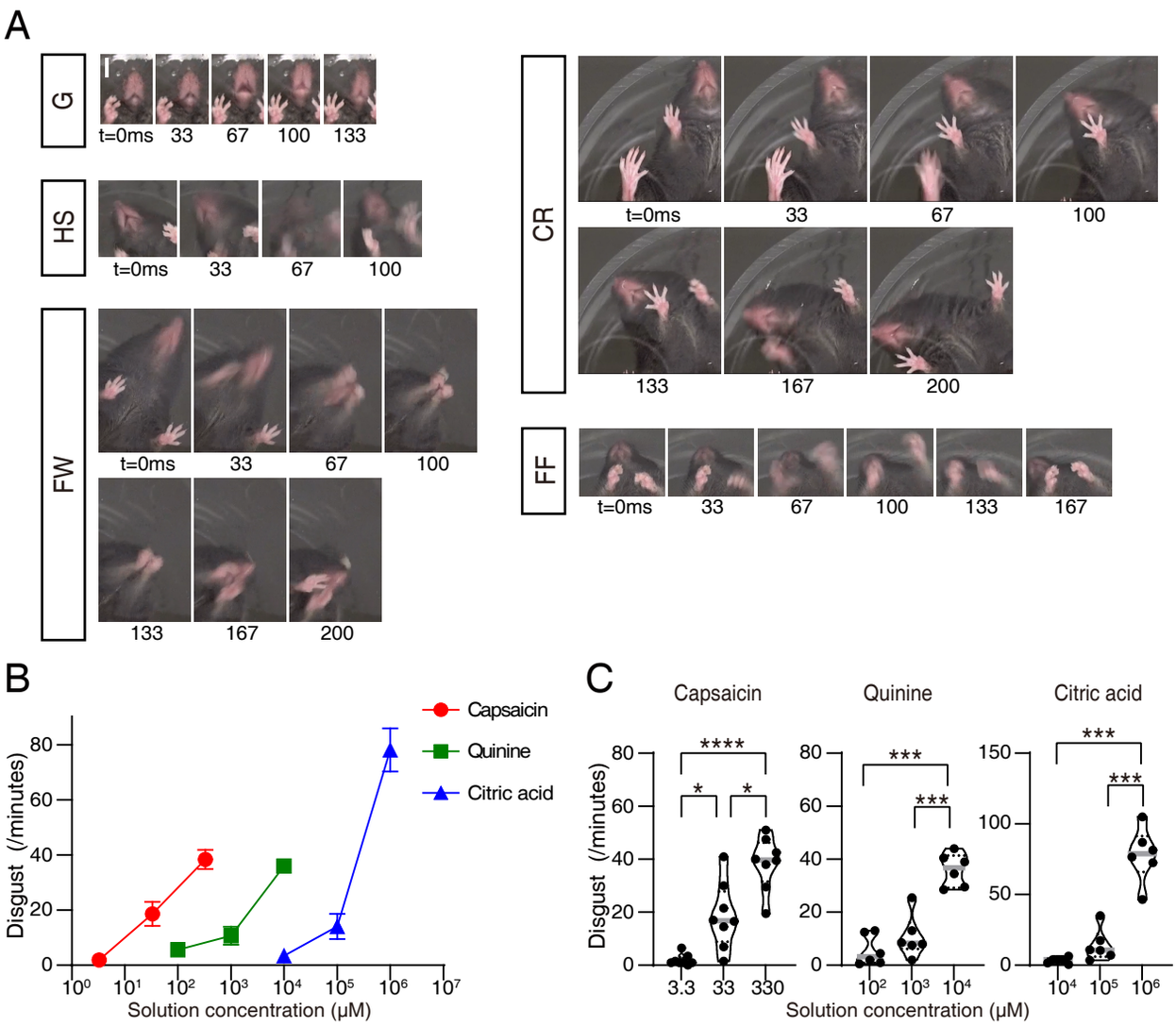
A
G
t=0ms 33 67 100 133
HS
t=0ms 33 67 100
FW
t=0ms 33 67 100
133 167 200
CR
t=0ms 33 67 100
133 167 200
FF
t=0ms 33 67 100 133 167
B
Capsaicin
Quinine
Citric acid
Disgust (/minutes)
Solution concentration (μM)
C
Capsaicin
Quinine
Citric acid
Disgust (/minutes)
Solution concentration (μM)

Figure 2

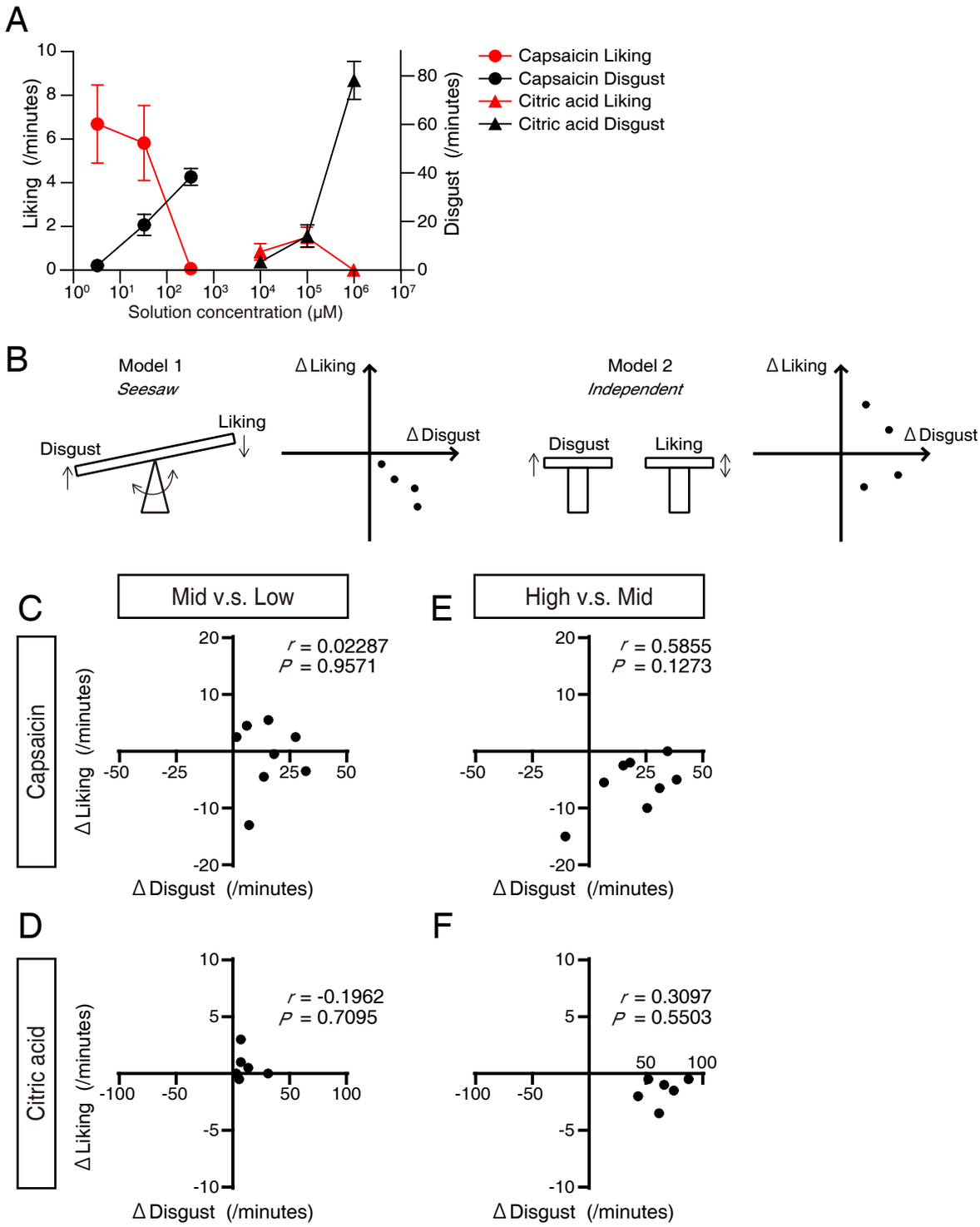
A
Liking (/minutes)
Disgust (/minutes)
Solution concentration (μM)
Capsaicin Liking
Capsaicin Disgust
Citric acid Liking
Citric acid Disgust
B
Model 1
Seesaw
Disgust
Liking
Δ Liking
Δ Disgust
Model 2
Independent
Disgust
Liking
Δ Liking
Δ Disgust
C
Mid v.s. Low
Capsaicin
r = 0.02287
P = 0.9571
Δ Liking (/minutes)
Δ Disgust (/minutes)
E
High v.s. Mid
r = 0.5855
P = 0.1273
Δ Disgust (/minutes)
D
Citric acid
r = -0.1962
P = 0.7095
Δ Liking (/minutes)
Δ Disgust (/minutes)
F
r = 0.3097
P = 0.5503
Δ Disgust (/minutes)

Figure 3

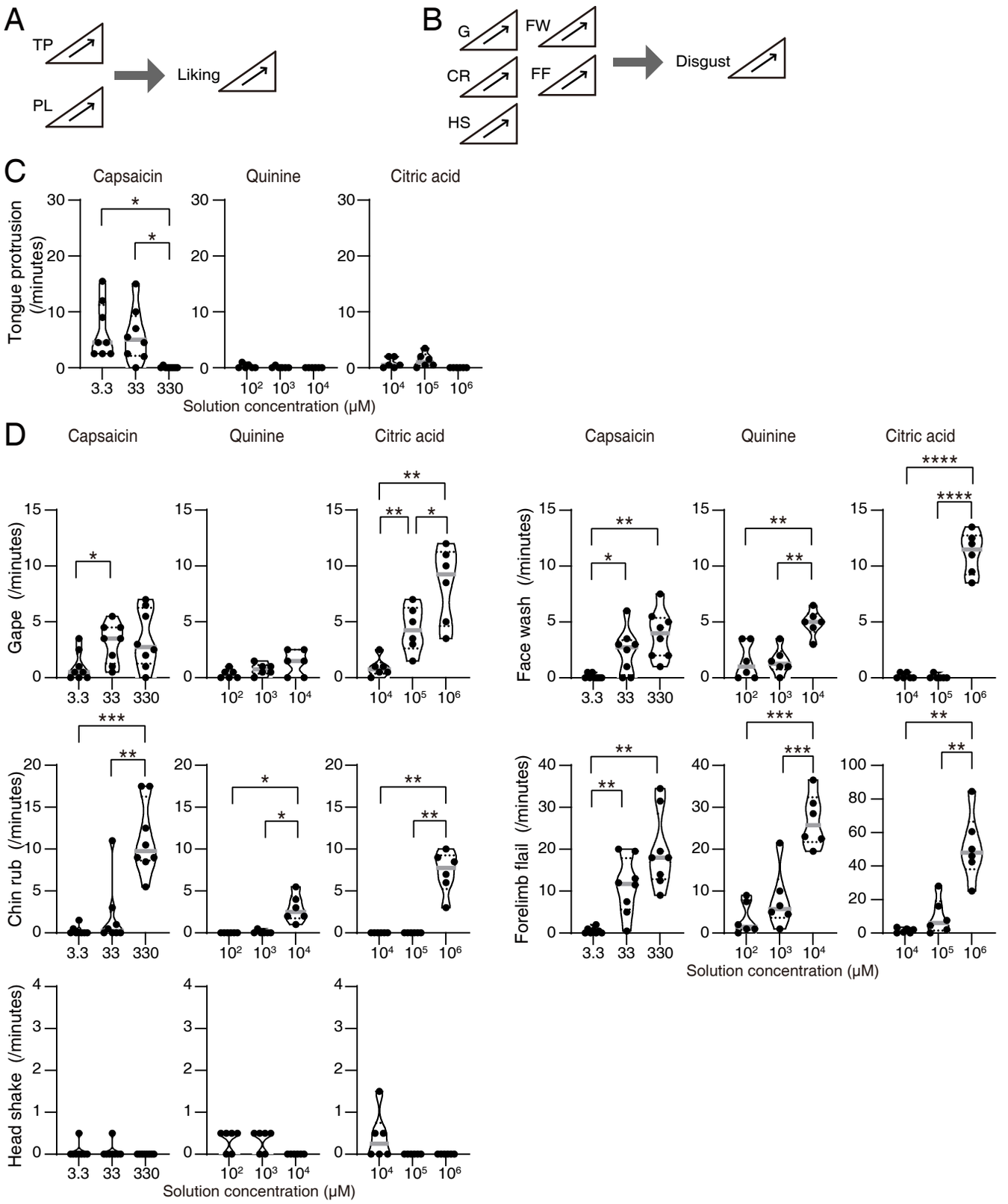
A
TP
PL
Liking
B
G
FW
CR
FF
HS
Disgust
C
Capsaicin
Quinine
Citric acid
Tongue protrusion (/minutes)
Solution concentration (μM)
D
Capsaicin
Quinine
Citric acid
Gape (/minutes)
Face wash (/minutes)
Chin rub (/minutes)
Forelimb flail (/minutes)
Head shake (/minutes)
Solution concentration (μM)

Figure 4

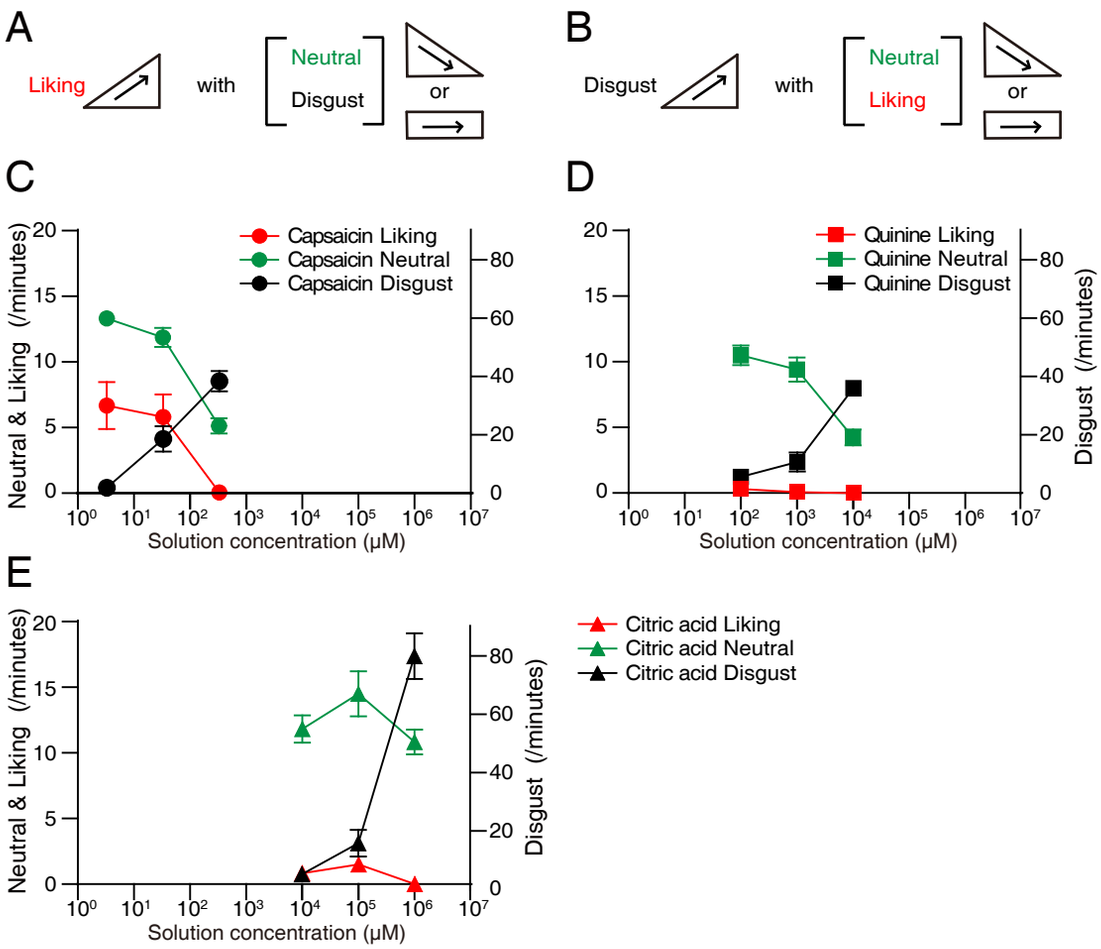
A
Liking
with
Neutral
Disgust
or
B
Disgust
with
Neutral
Liking
or
C
Capsaicin Liking
Capsaicin Neutral
Capsaicin Disgust
Neutral & Liking (/minutes)
Solution concentration (μM)
D
Quinine Liking
Quinine Neutral
Quinine Disgust
Disgust (/minutes)
Solution concentration (μM)
E
Citric acid Liking
Citric acid Neutral
Citric acid Disgust
Neutral & Liking (/minutes)
Disgust (/minutes)
Solution concentration (μM)

Figure 5

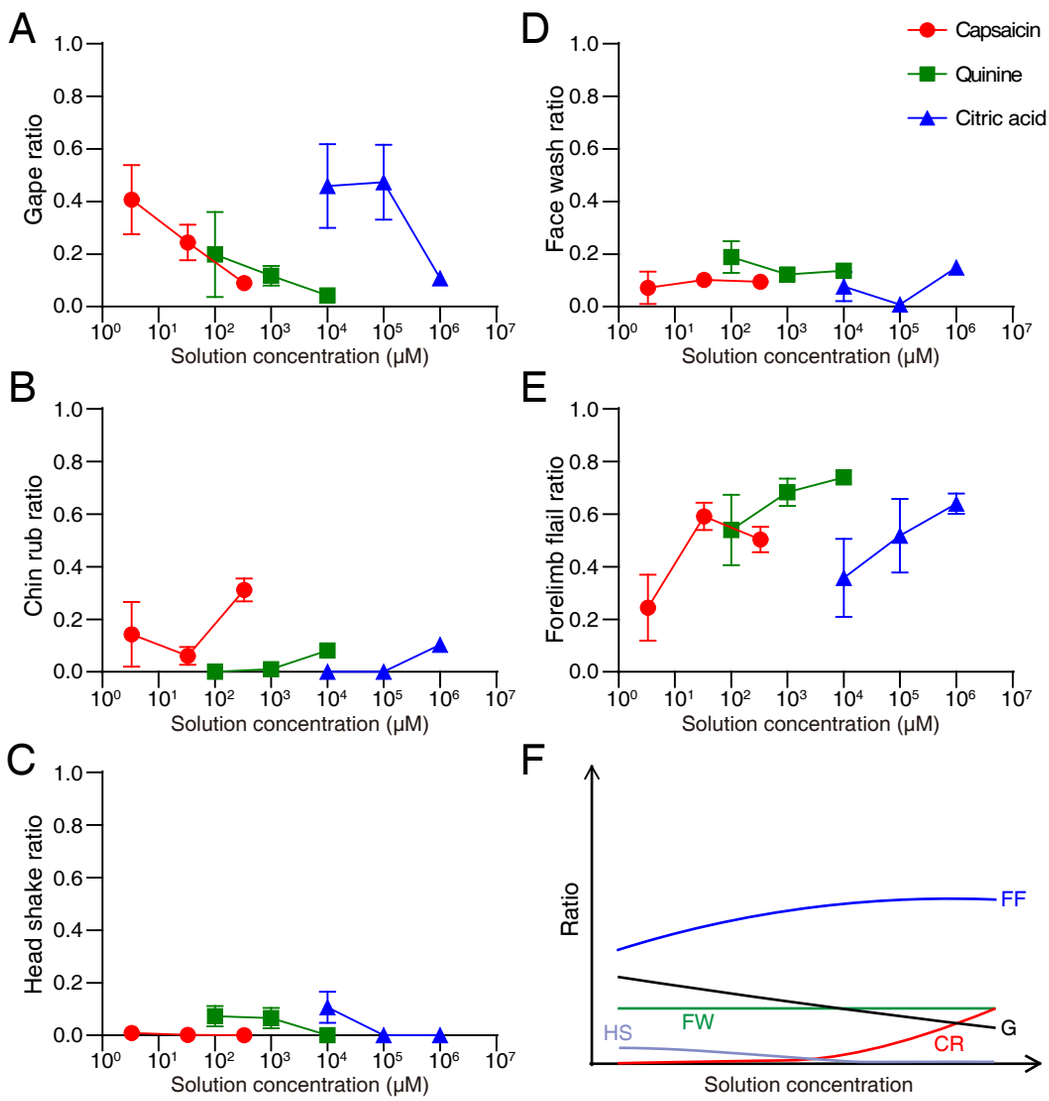
A
Gape ratio
Solution concentration (μM)
B
Chin rub ratio
Solution concentration (μM)
C
Head shake ratio
Solution concentration (μM)
D
Face wash ratio
Solution concentration (μM)
Capsaicin
Quinine
Citric acid
E
Forelimb flail ratio
Solution concentration (μM)
F
Ratio
FF
FW
HS
G
CR
Solution concentration

Figure 6