Ammonia and its amine-containing derivatives are widely found in natural decomposition byproducts. Here, we conducted biased chemoreceptor screening to investigate the mechanisms by which different concentrations of ammonium salt, urea, and putrescine in rotten fruits affect feeding and oviposition behavior. We identified three ionotropic receptors, including the two broadly required IR25a and IR76b receptors, as well as the narrowly tuned IR51b receptor. These three IRs were fundamental in eliciting avoidance against nitrogenous waste products, which is mediated by bitter-sensing gustatory receptor neurons (GRNs). The aversion of nitrogenous wastes was evaluated by the cellular requirement by expressing Kir2.1 and behavioral recoveries of the mutants in bitter-sensing GRNs. Furthermore, by conducting electrophysiology assays, we confirmed that ammonia compounds are aversive in taste as they directly activated bitter-sensing GRNs. Therefore, our findings provide insights into the ecological roles of IRs as a means to detect and avoid toxic nitrogenous waste products in nature.
Nitrogen is an essential building block for the synthesis of DNA and protein and is the most abundant element in the Earth’s atmosphere. Therefore, the nitrogen cycle is instrumental in maintaining healthy ecosystem dynamics. Animals and many plants produce nitrogenous wastes throughout their life histories. Ammonia and urea are the decomposition byproducts of protein, whereas rotten fruits are rich in polyamines such as putrescine-4. These organic compounds can be recycled as a source of nitrogen groups such as amines, amidines, and anilines, which are central for developmental and physiological processes in both plants and animals. In nature, ammonia concentration is in the range of 10–20 mM in the cassava plant leaf and the cattle manure, among others. Animals, including insects, rely on chemoreception for feeding, mating, and escaping from predators. Moreover, although the anatomy and molecular basis of taste perception in vertebrates and invertebrates are evolutionarily distinct, they share a few similar fundamentals. Fruit flies (Drosophila melanogaster) possess specialized taste neurons on their labella, legs, pharynx, wing margins, and ovipositor. Flies can sense sweetness, bitterness, sourness, saltiness, and water. Major gustatory organs, such as the labellum and legs, have evolved to recognize chemicals via several channels and receptors, such as gustatory receptors (GRs), odorant receptors (ORs), ionotropic receptors (IRs), transient receptor potential (TRP) channels, and pick-pocket ion channels (PPPKs). Most taste sensilla harbor four distinct GRNs, of which two are attractive GRNs (sweet-sensing and water-sensing GRNs) and two are aversive GRNs (bitter-sensing or calcium-sensing GRNs). Moreover, these neuronal circuits have been found to be distinct, as attractive or aversive GRNs can be independently activated and behaviorally controlled by artificially expressing temperature-activated TRPA1, capsaicin-activated rat TRPV1, or light-activated channelrhodopsin.

Among these chemoreceptors, IRs are broadly expressed in the peripheral sensory systems involved in chemosensation, thermosensation, and hygroreception. Recent studies on the mechanisms of taste perception indicate that saltiness, sourness, amino acids, and other chemical cues are directly sensed by taste IRs. In nature, amine-containing compounds not only elicit aversive responses but have also been identified as important kairomones in host-seeking insects. For instance, insects and some disease vectors are attracted by the odor of ammonia and ammonium chloride strongly activate S5, S6, and S7 sensilla but not L5- or I-type sensilla. Flies avoided ammonia-containing food. This made us measure the pH of each chemical. Ammonium sulfate and ammonium chloride strongly activate S5, S6, and S7 sensilla but not L5- or I-type sensilla, indicating that these nitroso compounds can be easily dried to generate much higher concentration in the wild condition. GRNs are mainly classified into four different categories. These GRNs include the sweet-sensing Gr5a-GALA, bitter-sensing Gr66a-GALA or Gr33a-GALA, calcium-sensing pkP23-GALA, and water-sensing pkP28-GALA neurons. Sugar and water are generally attractive, whereas bitterness and calcium are aversive. To perform an unbiased test, we expressed the inwardly rectifying potassium channel (Kir2.1) gene to inhibit each category. All four chemicals induced action potentials in the S6 sensilla of the controls (w1118 and UAS-Kir2.1/+). These action potentials were only inhibited by the ablation of bitter-sensing GRNs (Gr33a-GALA/UAS-Kir2.1 and Gr66a-GALA/UAS-Kir2.1), whereas the other neurons exhibited similar responses to those of the controls. The results of these electrophysiological tip recordings were further confirmed by our behavioral assays. Flies avoided ammonium sulfate, ammonium chloride, urea, and putrescine in a dose-dependent manner. Female flies also avoided laying their eggs on surfaces containing 50 mM of each chemical, and this response was mediated by bitter-sensing GRNs. Female flies laid slightly more eggs on ammonia-containing food. This made us measure the pH of each chemical. Ammonium sulfate (pH 5.8), ammonium chloride (pH 5.9), and putrescine (pH 5.5) are slightly acidic in solution, whereas urea (pH 7.6) is slightly basic. To address whether the pH affected oviposition behavior and binary food choice assay, we tested ammonium sulfate which were adjusted to neutral pH by adding ammonium hydroxide (Supplementary Fig. 1d, e). The aversions of wild-type flies were not affected by the slight change of pH. Next, the slight attraction of bitter-sensing GRNs-ablated flies to the chemicals in oviposition may be affected by olfaction, because a population of OR neurons and projection neurons are activated by ammonia. To test any possible roles of antenna on oviposition, we tested normal and antenna-removed female flies with the same genotypes. We found that antenna-removed controls and Gr66a-GALA/UAS-Kir2.1 flies showed slightly increased oviposition index, but not statistically significant, compared to normal flies (Supplementary Fig. 1f). These results confirmed that the bitter-
sensing GRNs in taste had a major role in affecting oviposition on the ammonia-containing media, though ammonia odor may partially influence female flies to select egg laying sites if any. Furthermore, to confirm the putative aversiveness of nitrogenous wastes and their role in activating bitter-sensing GRNs, the response of flies to each chemical was characterized via an adaptation of the proboscis extension reflex (PER) assay. This PER response was evaluated after a pre-stimulus with sucrose (Fig. 1f). As expected, this paradigm provided cellular-level evidence of the involvement of bitter-sensing GRNs, but not calcium-sensing GRNs, in the aversive response to nitrogenous waste products.
Further, we performed labellar tip recordings with 100 mM ammonium sulfate using candidate IRs to identify the molecular basis of ammonium sulfate, ammonium chloride, urea, and putrescine perception (Fig. 2a). We found deficits in three mutants of the broadly required IR25a and IR76b receptors, in addition to a newly identified IR51b mutant from 28 Ir mutants, which were expressed in the labellum and taste sensilla that surround the legs and wing margins (Fig. 2a and Supplementary Table 1). Tip recordings of the response of other candidate GR mutants to ammonium sulfate were conducted to further confirm the results of our screening experiments (Supplementary Fig. 2a). Once again, we found that only three IRs were required for ammonium sulfate perception. We also tested neutral ammonium sulfate for control and three IR mutants in electrophysiology (Supplementary Fig. 2b). We confirmed that the pH of the ammonia had no role in the residual spikes in mutant flies.

Next, tip recordings were conducted to assess the responses to the remaining chemicals. Using a 0–100 mM range, we found that the same IRs are required for sensing ammonium chloride, urea, and putrescine (Fig. 2b–d). To further support the indispensable role of these three IRs to respond to nitrogenous wastes, rescue experiments were conducted using a targeted gene expression approach via the GAL4/UAS system. These genetic experiments indicated that the deficits of Ir25a2 and Ir76b1 mutants to sense ammonium sulfate, urea, and putrescine were recovered through the expression of each wild-type cDNA under the control of the indicated GAL4 promoters. Action potentials were elicited on S6 sensilla from the labellum with 100 mM of (NH4)2SO4, CO(NH2)2, and NH2(CH2)4NH2 (n = 10–12). All error bars represent the SEMs. Multiple comparisons were conducted using single-factor ANOVA coupled with Scheffe’s post hoc test. The colored asterisks in panels (e–g) indicate a significant difference between the same-colored bars compared to the control (**P < 0.01).

![Fig. 2 Three IRs are required for the detection of nitrogenous wastes, as determined by electrophysiology assays. a Average number of spikes per second when the S6 sensilla on the labellum were stimulated with 100 mM (NH4)2SO4 [control (w1118) and 28 Ir mutant lines] (n = 10). b–d Average frequencies of action potentials obtained by performing tip recordings on the S6 sensilla of the control (w1118) and three candidate Ir mutant flies in responses to the indicated doses (mM) of (b) ammonium salts, (c) CO(NH2)2, and (d) NH2(CH2)4NH2, respectively (n = 10–11). e–g Rescue of tip recording defects in (e) Ir25a2, (f) Ir76b1, (g) Ir51b1 mutants by expressing the respective wild-type cDNA under the control of the indicated GAL4 promoters. Action potentials were elicited on S6 sensilla from the labellum with 100 mM of (NH4)2SO4, CO(NH2)2, and NH2(CH2)4NH2 (n = 10–12). All error bars represent the SEMs. Multiple comparisons were conducted using single-factor ANOVA coupled with Scheffe’s post hoc test. The colored asterisks in panels (e–g) indicate a significant difference between the same-colored bars compared to the control (**P < 0.01).](https://doi.org/10.1038/s42003-021-02799-3)
Gr33a-GAL4 bitter-sensing GRNs and its respective GAL4, as demonstrated by our electrophysiology experiments (Fig. 2e, f). Furthermore, we rescued the Ir51b deficit by expressing Ir51b cDNA under the control of Ir25a-GAL4 or Gr33a-GAL4 (Fig. 2g).

To assess the molecular basis of ammonium-induced aversive behaviors, the response of the candidate IRs Ir25a, Ir51b, and Ir76b to a 100 mM sucrose solution versus 100 mM sucrose + 50 mM ammonium sulfate were examined via two-way choice feeding assays (Fig. 3a). The results of these behavioral assays supported the physiological evidence that mutations in Ir25a, Ir51b, and Ir76b only resulted in diminished avoidance to 50 mM ammonium sulfate, whereas mutation of other Ir5 had no significant effect on ammonium sulfate avoidance in the feeding assay. Three IRs may also affect olfaction, while taste is likely a...
Fig. 3 Three IRs are required to avoid toxic concentrations of nitrogenous waste products. a Screening of Ir mutants using the binary food choice assay. The flies were given a choice between 100 mM sucrose versus 100 mM sucrose laced with 50 mM (NH₄)₂SO₄ (n = 6). b, c Binary food choice assay results showing the rescue of feeding defects in ir25a² mutants in response to (b) 50 mM (NH₄)₂SO₄, 50 mM CO(NH₂)₂, and (c) 50 mM NH₃(CH₂)₄NH₃ when wild-type Ir25a cDNA was expressed under the control of Ir25a-GAL4 as well as Gr33a-GAL4 (n = 6–11). d, e Behavioral rescue of feeding defects in Ir76b mutants exposed to (d) 50 mM (NH₄)₂SO₄, 50 mM CO(NH₂)₂, and (e) 50 mM NH₃(CH₂)₄NH₃ expressing wild-type Ir76b cDNA under the control of Ir76a-GAL4 or Gr33a-GAL4 (n = 6–11). f, g Binary food choice assays to assess the rescue the feeding defects of Ir51b mutants in response to (f) 50 mM (NH₄)₂SO₄, 50 mM CO(NH₂)₂, and (g) 50 mM NH₃(CH₂)₄NH₃ when wild-type Ir51b cDNA was driven under the control of Ir25a-GAL4 or Gr33a-GAL4 (n = 6–10). All error bars represent the SEMs. Multiple comparisons were conducted using single-factor ANOVA coupled with Scheffe’s post hoc test. The colored asterisks indicate statistical significance with the same-colored bar values compared to the control (*P < 0.01).

Fig. 4 Ir51b is expressed in bitter-sensing GRNs on the labellum. a Gel picture of RT-PCR results showing Ir51b expression in the labellum and legs, but not in the antennae. Amplified tubulin products were used as control. “M” indicates the DNA ladder marker. b Quantification of Ir51b RNA levels which was normalized by tubulin in the same reaction in panel (a). The density of Ir51b RNA was divided by tubulin RNA level in each batch and control (whole body) was set to 100 by same fold change in the batch. Three repeated experiments were provided. c Gel picture of Ir51b expressed in bitter-sensing GRNs. The 1.7 kb Ir51b gene was amplified using RT-PCR in no-DNA template, the control (w¹¹¹⁸), UAS-hid, Gr5a-GAL4 (sugar-sensing GRNs), Gr33a-GAL4 (bitter-sensing GRNs), Gr5a-GAL4/UAS-hid (sugar-sensing GRNs ablated), and Gr33a-GAL4/UAS-hid (bitter-sensing GRNs ablated) flies. Amplified tubulin products were used as internal control of PCR reaction. “M” indicates the DNA ladder marker. d Quantification of Ir51b RNA levels which was normalized by tubulin in the same reaction in panel (c). Three repeated experiments were provided. The density of Ir51b RNA was divided by tubulin RNA level in each batch and control (w¹¹¹⁸) was set to 100 by same fold change in the batch. All error bars represent the SEMs. Multiple comparisons were conducted using single-factor ANOVA coupled with Scheffe’s post hoc test. The asterisks indicate statistical significance, compared to the control (whole-body sample in (b) and control in (d)) (**P < 0.01).

The main determinant of ammonia aversion in feeding behavior which is highly attributed to changes in taste physiology. Similar to the electrophysiological responses, we confirmed that the same Ir mutants exhibited behavioral responses to ammonium sulfate, urea, and putrescine concentrations ranging from 0 to 75 mM (Supplementary Fig. 3a–c). Additionally, upon rescuing the deficits of these mutants via genetic experiments, the results of our behavior assays were consistent with those of our electrophysiology recordings (Fig. 3b–g).

We previously reported that IR25a was co-expressed extensively with the IR76b reporter²⁹. Further, a previous study indicated that the Ir51b-GAL4 reporter was not detected in the labellum¹⁵. In addition, Ir51b is detected in the RNA-seq analysis of the Drosophila antenna¹⁶. To investigate whether Ir51b RNA is expressed in the labellum, we performed reverse transcriptase-polymerase chain reaction (RT-PCR) using labellum, leg, and antenna samples (Fig. 4a and Supplementary Fig. 5a). We identified the predicted 1.7 kb band in the wild-type labella and legs, but not antennae. This band was confirmed by DNA sequencing. Furthermore, repeated experiments for three independent sample preparation were quantified by ImageJ and normalization (Fig. 4b). This suggests that IR51b acts as a contact-mediated chemosensor. However, we do not completely exclude any possible role of IR51b in antenna, because other group detects low-level expression of Ir51b by RNA-seq. Next, we expressed the cell death gene UAS-hid under the control of sweet-sensing (Gr5a-GAL4) or bitter-sensing (Gr33a-GAL4) receptors to detect whether Ir51b participates in the GRN-mediated perception of bitter substances using tubulin as an internal control (Fig. 4c, d and Supplementary Fig. 5b). We found that Ir51b RNA was almost completely eliminated in the Gr33a-GAL4/UAS-hid mutants (Fig. 4c, d and Supplementary Fig. 5b). Next, we generated a second Ir51b allele using ends-out homologous recombination (Supplementary Fig. 4). The in-frame knock-in of GAL4 was generated with a 1017 bp deletion of the exon; however, we failed to recapitulate the expression pattern of Ir51b. Therefore,
this mutant was named Ir51b\textsuperscript{2}. This second allele also showed similar deficits to those of Ir51b\textsuperscript{1} (Fig. 2b–d), thus suggesting the role of IR51b in the GRN-mediated perception of bitter-tasting (aversive) compounds.

To investigate the genetic recapitulation of ammonia-taste receptors, we assessed whether these three genes were sufficient to elicit taste-induced avoidance of nitrogenous compounds in flies. I-type sensilla in the fly’s labellum possess only two GRNs, whereas L-type and S-type sensilla harbor four GRNs (Fig. 5a–c). We then induced the expression of wild-type IR51b cDNA in the bitter-sensing I-type cells or sweet-sensing L-type cells where they are normally not expressed based on our mapping results (Supplementary Fig. 1a). The action potentials of the I-type sensilla of the indicated genotypes with 100 mM (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} (n = 20) were recorded in bitter-sensing GRNs under the control of Gr33a-GAL4. Average action potentials were generated on I8 and I9 sensilla from the labellum of the indicated genotypes with 100 mM (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} (n = 20). e Representative sample traces from panel (d). f Overexpression of Ir25a, Ir51b, and Ir76b cDNA in sugar-sensing GRNs in L-type sensilla under the control of Gr33a-GAL4. Average action potentials were generated on L4 and L6 sensilla in the labellum of the indicated flies with 100 mM (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} (n = 20). All error bars represent SEMs. Multiple comparisons were conducted using single-factor ANOVA coupled with Scheffe’s post hoc test (\textsuperscript{p} < 0.01).

Future works should focus on finding additional channel subunits to prove that nitrogenous waste compounds can directly activate these IRs by heterologous expression. Chemical sensation is an essential modulator of physiology and behavior. In invertebrates, the vast majority of chemical stimuli in the environment are recognized by members of two evolutionarily related chemosensory receptors: the ORs and the GRs\textsuperscript{51}. However, recent studies have indicated that IRs are likely the most ancient chemoreceptors and thus predate ORs and GRs, as their existence can be traced back prior to the deuterostome-protostome split\textsuperscript{16,27}.

Fig. 5 Overexpression of IR25a, IR51b, and IR76b in bitter-sensing or sugar-sensing GRNs. a–c Cartoons of three different types of gustatory sensilla and their GRNs on the labellum of Drosophila. a Heteromeric association of ir25a, ir51b, and ir76b in S-type sensilla for ammonia taste processing. b Misexpression of ir51b cDNA in I-type sensilla. c Misexpression of ir51b cDNA in L-type sensilla. d UAS-Ir51b alone or UAS-Ir25a, UAS-Ir51b, and UAS-Ir76b were expressed in bitter-sensing GRNs under the control of Gr33a-GAL4. Average action potentials were generated on I8 and I9 sensilla from the labellum of the indicated genotypes with 100 mM (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} (n = 20). e Representative sample traces from panel (d). f Overexpression of Ir25a, Ir51b, and Ir76b cDNA in sugar-sensing GRNs in L-type sensilla under the control of Gr3a-GAL4. Recordings of nerve responses were performed on L4 and L6 sensilla in the labellum of the indicated flies with 100 mM (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} (n = 20). All error bars represent SEMs. Multiple comparisons were conducted using single-factor ANOVA coupled with Scheffe’s post hoc test (\textsuperscript{p} < 0.01).
Ammonia can act as a kairomone, and therefore some species, such as flour mites, are attracted to the microbial degradation products of certain amino acids52. Females of some urticating muscid flies are reportedly attracted to ammonia when searching for suitable oviposition sites53. In this case, ammonia acts as a chemical attractant that enables some parasitic organisms to detect their hosts. However, ammonia can also be used to kill or repel bed bugs, ants, rats, fleas, and snakes. Insect survival may also vary depending on ecological niche or host characteristics; however, the proliferation of insect populations is generally thought to be highly host-dependent. Here, we demonstrated that fruit flies avoided nitrogenous waste products both when laying eggs and when selecting their food, as these compounds are potentially toxic. Despite the differences in the mechanisms of chemical sensation between arthropods and humans, the identification of ammonia-associated taste sensors in insects provides important insights into how animals perceive and react to specific chemicals.

Methods

Fly strains. Unless otherwise indicated, all flies were maintained at 25 °C under a 12:12 h light/ dark cycle. Both male and female flies were used randomly in our experiments. The control strain used in this study was w1118. We previously described the Ir51a1, Ir51a2, Ir51a5a, Ir60b1, Ir94a1, Ir94c1, and Ir51b1 strains50. The Ir51b2 (BL42402), Ir51b3 (BL23173), Ir51b4 (BL25638), and Ir51b5 (BL24205) strains were created via ends-out homologous recombination as previously described61. Community Ribeiro et al. created the mutant lines through PCR and subcloned the DNA into the pw35-GAL4 vector48. Right arm extension included 3050 bp and left arm extension included 2944 bp along with 21 bp flanking sequences. Labellum, leg, and antenna samples were dissected from approximately 100 flies. A reference glass electrode with Ringer’s solution (3 mM CaCl2, 182 mM KCl, 46 mM NaCl, and 10 mM Tris-base; pH 7.2) was inserted into the thorax of the flies. The glass electrode was gently pushed towards their proboscis without causing any severe damage to the GRNs on the proboscis. Approxi mately 4–6 flies were used for each experiment. Using an electrophysiology system, we activated the S-type, I-type, and L-type taste sensilla on the labella of flies for 5 s using a mixture of hydrocarbons with 30 mM triclinic citrate (TCC). The recording electrode (10–20 μm tip diameter) was connected to a preamplifier (Taste PROBE, Syntech, Hilversum, The Netherlands), and the signals were collected and amplified by 10x using a signal connection interface box (Syntech) in conjunction with a 100–3000 Hz band-pass filter. Recordings of action potentials were acquired using a 12 kHz sampling rate and analyzed using the Autospike 3.1 software (Syntech). We then counted the action potentials for 50–550 ms and presented doubled values of the period per second in the figures. Each consecutive recording was performed with an approximately 1 min gap between each stimulation. The sample numbers (n) in each experiment indicate the number of animals. The same procedure was repeated on different days and using different setups.

Probiotic extension reflex (PER) assay. The PER assay was performed as previously described54. The flies were first starved for 20–24 h in a vial with water-soaked Kimwipe paper. The flies were then briefly anesthetized on ice and fixed on a glass slide using glue. A fine tungsten wire was then used to deliver the initial 100 μM sucrose stimulus to the flies. Only flies that showed a positive PER to sucrose were considered for the next test. Taste stimuli were delivered to the labellum at least three times to avoid false-positive responses. At this point, the flies that exhibited a positive PER to the experimental solutions (i.e., 100 mM ammonia in 100 mM sucrose) were deemed PER positive. A total of 10–15 flies were evaluated per experiment, after which PER percentages were calculated. At least six replicates were performed for each strain.

Oviposition preference assay. Oviposition preference assays were conducted as described in a previous study55. A total of 15 female and 15 male newly hatched flies were transferred into a new food vial supplemented with dry yeast and kept in a normal light-dark cycle for two days. Prior to the assays, the experimental animals were acclimatized in 1% agarose containing a test food choice for 5–6 h. Two food options were then provided, one containing only sucrose and another containing a mixture of sucrose and a nitrogen-containing chemical, both of which were dispensed on a Petri dish (35 mm diameter, Product No. 351007) divided into two equal halves. The agarose food was allowed to solidify and then transferred to an egg-laying chamber (Code No. FEC-50200, Hansol Tech, Republic of Korea); the acclimated flies were transferred into the chamber thereafter. The flies were then allowed to lay eggs overnight inside of the incubator. The next day, the number of eggs deposited on each side of the chamber (i.e., each containing a different food option) was counted, and the ovipositional preference index was calculated as follows:

\[
\text{PI} = \frac{(N_{\text{red}} - N_{\text{blue}})}{(N_{\text{red}} + N_{\text{blue}} + N_{\text{purple}})} \quad \text{or} \quad \text{PI} = \frac{(N_{\text{blue}} - N_{\text{red}})}{(N_{\text{red}} + N_{\text{blue}} + N_{\text{purple}})}
\]

depending on the dye/tastant combinations. At least six replicates were performed for each fly strain.

Electrophysiology. Tip recording assays were conducted as described in a previous study56. Two-way food choice assay. Two-way food choice assays were performed as described in a previous study57. First, 5–7-day-old mixed gender (males and females were randomly selected) flies were starved in a vial containing water-soaked Kimwipe paper for 16–18 h in a dark and humid environment. Each experiment was conducted using 50–70 flies. We then prepared two food options, both containing 1% agarose: one contained only sucrose and the other contained sucrose mixed with nitrogen-containing chemicals. These food sources were colored with either blue (brilliant blue FCF, 0.125 mg/mL) or red food-grade dye (sulfonohoma mine B, 0.1 mL/mL). These two food preparations were dispensed into a 7-well microtiter dish (Thermo Fisher Scientific, Cat No 438733) in an alternative position. We briefly anesthetized the starved flies and introduced them into the food dish, after which we immediately transferred them to an incubator for 90 min. The flies were euthanized in a –20 °C freezer for at least 2 h. Then, the abdomen color was classified as “blue,” “red,” or “purple” using a stereomicroscope. The preference index (PI) was calculated using following equations:

\[
\text{PI} = \frac{(N_{\text{red}} - N_{\text{blue}})}{(N_{\text{red}} + N_{\text{blue}} + N_{\text{purple}})} \quad \text{or} \quad \text{PI} = \frac{(N_{\text{blue}} - N_{\text{red}})}{(N_{\text{red}} + N_{\text{blue}} + N_{\text{purple}})}
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\text{PI} = \frac{(N_{\text{red}} - N_{\text{blue}})}{(N_{\text{red}} + N_{\text{blue}} + N_{\text{purple}})} \quad \text{or} \quad \text{PI} = \frac{(N_{\text{blue}} - N_{\text{red}})}{(N_{\text{red}} + N_{\text{blue}} + N_{\text{purple}})}
\]

depending on the dye/tastant combinations. At least six replicates were performed for each fly strain.
represent the standard error of the mean (SEM). Multiple comparisons were then evaluated using single-factor ANOVA coupled with Scheffe’s post hoc test. Asterisks indicate statistical significance. (P < 0.05, **P < 0.01). Statistical analyses were performed using Origin Pro 8 for Windows (ver. 8.032; Origin Lab Corporation, USA).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
The datasets used in this paper are preserved with corresponding author, which are available upon reasonable request. The source data for the individual values and scripts used to generate figures are attached to this paper as Supplementary Data 1.

Received: 27 April 2021; Accepted: 19 October 2021;
Published online: 12 November 2021.

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Acknowledgements
This work was supported by grants to Y.L. from the Basic Science Research Program of the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2018R1A2B6004202 and NRF-2021R1A2C1007628) and the Korea Environmental Industry and Technology Institute (KEITI) grant funded by the Ministry of Environment of Korea. S.D. and B.A. were supported by the Global Scholarship Program for Foreign Graduate Students at Kookmin University in Korea.

Author contributions
Y.L. designed the research. S.D. performed the majority of research and analyzed the data. J.S. and B.A. performed important behavioral and electrophysiological experiments and analyzed. Y.L. commented on the results. S.D. and Y.L. combined the results and wrote the paper.

Competing interests
The authors declare no competing interests.

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Peer review information Communications Biology thanks Makoto Hiroi and the other, anonymous, reviewers for their contribution to the peer review of this work. Primary handling editor: Caitlin Karniski. Peer reviewer reports are available.

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