LETTER

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Bees prefer foods containing neonicotinoid pesticides

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The impact of neonicotinoid insecticides on insect pollinators is highly controversial. Sublethal concentrations alter the behaviour of social bees and reduce survival of entire colonies[1–3]. However, critics argue that the reported negative effects only arise from neonicotinoid concentrations that are greater than those found in the nectar and pollen of pesticide-treated plants[4]. Furthermore, it has been suggested that bees could choose to forage on other available flowers and hence avoid or dilute exposure[4,5]. Here, using a two-choice feeding assay, we show that the honeybee, *Apis mellifera*, and the buff-tailed bumblebee, *Bombus terrestris*, do not avoid nectar-relevant concentrations of three of the most commonly used neonicotinoids, imidacloprid (IMD), thiamethoxam (TMX), and clothianidin (CLO), in food. Moreover, bees of both species prefer to eat more of sucrose solutions laced with IMD or TMX than sucrose alone. Stimulation with IMD, TMX and CLO neither elicited spiking responses from gustatory neurons in the bees' mouthparts, nor inhibited the responses of sucrose-sensitive neurons. Our data indicate that bees cannot taste neonicotinoids and are not repelled by them. Instead, bees preferred solutions containing IMD or TMX, even though the consumption of these pesticides caused them to eat less food overall. This work shows that bees cannot control their exposure to neonicotinoids in food and implies that treating flowering crops with IMD and TMX presents a sizeable hazard to foraging bees.

Determining the impacts of pesticides on pollinators is important to resolve for the future of world food security. Pollinating insects like bees increase the yields of human crops, but in doing so, are inadvertently exposed to pesticides in floral nectar and pollen[6,7]. Several studies have concluded that bees exposed to sublethal doses of neonicotinoid pesticides in food have difficulty learning floral traits, feeding, navigating and foraging[2,3,8–11], and have impaired motor function[12]. These changes in behaviour often lead to colony failure[2,3]. This body of work has galvanized public concern over bee welfare, and in 2013, led to a two-year ban on the use of the three most common neonicotinoids (IMD, TMX, CLO) on flowering crops by the European Union. The agricultural importance of these pesticides has motivated agrochemical producers and government scientists to challenge this ban. Critics of laboratory-based experiments contend that such studies use food laced with neonicotinoid concentrations that exceed the levels found in nectar and pollen[13], or give bees no choice of food solutions[4,5]. They propose that free-living bees and other insect pollinators could choose to avoid the nectar and pollen of pesticide-treated crops[4] if pollinators are repelled by neonicotinoids[14,15], and if alternative sources were provided such as field margins in agricultural settings.

These arguments require that pollinators are able to detect neonicotinoids in food in order to avoid exposure. We tested whether bees avoid sucrose solutions (that is, nectar) containing neonicotinoids using a two-choice test designed to identify the bumblebee's gustatory detection thresholds for nectar toxins[16]. Individual foraging-age worker bumblebees or cohorts of 25 forager honeybees were housed in plastic boxes for 24 h and given access to two types of food tubes: one containing sucrose solution and one containing sucrose solution laced with a specific concentration of the IMD, TMX or CLO. The concentrations used included values in the range reported from nectar and pollen (0.5–150 nM, Extended Data Table 1). Neither bumblebees nor honeybees avoided concentrations found within the naturally occurring range (Fig. 1a, b), even though high concentrations of TMX and CLO reduced their survival (Extended Data Fig. 1). We also tested whether these pesticides inhibited the honeybee's feeding reflex (proboscis extension) or caused honeybees to retract the proboscis once extended[17]. None of the sucrose solutions containing IMD, TMX or CLO affected proboscis extension or retraction (Extended Data Fig. 2).

Unexpectedly, we observed that both bumblebees and honeybees showed a preference for solutions containing IMD or TMX over sucrose alone (Fig. 1, Extended Data Tables 2, 3). Concentrations of IMD and TMX proximate to those found in nectar (1–10 nM, Extended Data Table 1) were most attractive to bumblebees (Fig. 1a), whereas honeybees preferred to consume IMD and TMX across a broader range of concentrations (Fig. 1b). The 'attractive' effect of IMD also depended on bee age: newly emerged adult worker bumblebees and honeybees largely avoided 1–10 nM IMD (Extended Data Fig. 3a). In addition, the presence of neonicotinoids influenced the total amount of food consumed from both tubes during 24 h (Fig. 1c, d). Bumblebees fed with IMD or CLO consumed less total food on average than those fed TMX or the sucrose control (Fig. 1c, Extended Data Table 2); this effect has also been observed by others[11,15]. In contrast, the total food consumption of forager honeybees was reduced only when bees fed from solutions containing 100 nM or 1 μM TMX or CLO (Fig. 2d, Extended Data Table 2). Thus, even in treatments where bees ate considerably less food in 24 h, they still preferred to consume solutions containing IMD over sucrose alone. Bumblebees also consumed 1.5–10-fold more of the neonicotinoid-laced food than honeybees and were, therefore, exposed to higher pesticide doses (Extended Data Table 4).

Insects detect nutrients and toxins in food via gustatory neurons in hair-like sensilla on the proboscis (mouthparts)[18]. Toxic, non-nutritious compounds elicit spikes in 'bitter'-sensing neurons[19,20], but can also be detected via suppression of the responses of sugar-sensing neurons[21,22]. Previous research has established that gustatory neurons located in sensilla on the honeybee's mouthparts are more sensitive to toxins in food[17] than its antennae[21] or tarsi[23]. If bees have mechanisms for detecting neonicotinoids, sensilla on the mouthparts should respond to these substances in the same way they respond to other toxins[17]. To test this, we recorded from gustatory neurons in sensilla on the galea (part of the proboscis) of bumblebees and honeybees using the tip recording technique (Fig. 2a, b). Stimulation with IMD, TMX or CLO in water did not elicit spikes from any of the neurons in the galeal sensilla of either bumblebees (Fig. 2c) or honeybees (Fig. 2d), whereas

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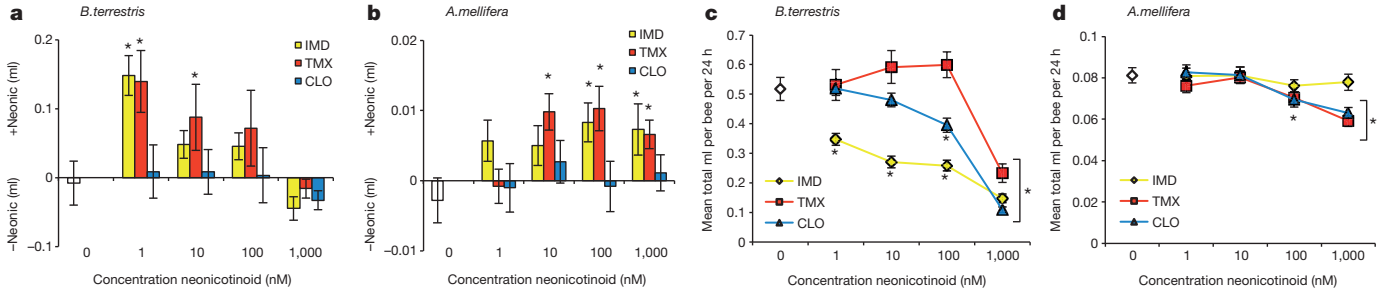

Figure 1 | Foraging-age bees prefer to eat food containing neonicotinoids. **a**, **b**, Bumblebees (**a**) and honeybees (**b**) given a choice of sucrose or sucrose containing a neonicotinoid pesticide chose to eat solutions containing IMD and TMX (Extended Data Table 2, bumblebees: generalized linear model (GLM): $\chi_2^2 = 12.1$, $P = 0.002$; honeybees: GLM, $\chi_2^2 = 11.1$, $P = 0.004$). Data represent the mean difference in the amount consumed over 24 h; positive values indicate a preference for solutions containing neonicotinoids. White bars indicate the sucrose control. Asterisks indicate $P \leq 0.002$ (Bonferroni-adjusted critical value) for one-sample *t*-tests against the '0' value (indicating no preference, see Extended Data Table 3). Sample sizes: bumblebees: IMD: 1 nM = 57, 10 nM = 66, 100 nM = 65, 1 μM = 66; TMX: 1 nM = 38, 10 nM = 39, 100 nM = 36, 1 μM = 40; CLO: 1 nM = 57, 10 nM = 59, 100 nM = 48, 1 μM = 62. Honeybees: $n = 40$ cohorts of 25 bees per treatment. Experiments were replicated with individuals taken from over 20 different bumblebee colonies and 4 honeybee colonies. **c**, The total amount of food eaten from both tubes by bumblebees was affected by the concentration and the presence of a neonicotinoid pesticide (GLM: $\chi_6^2 = 47.7$, $P < 0.001$, Extended Data Table 2) in one of the food tubes. **d**, Honeybees ate less total food only when it contained 1,000 nM TMX or CLO (GLM: $\chi_2^2 = 10.5$, $P = 0.005$, Extended Data Table 2). White diamonds indicate amount eaten by sucrose control group. $^*P < 0.05$ in post hoc comparisons against sucrose. Error bars represent ± s.e.m.

stimulation with nicotine hydrogen tartrate (NHT), KCl and sucrose did (Fig. 2c–f). This effect was the same for all three neonicotinoids in both bee species (Extended Data Table 5). To test whether neonicotinoids are detected via suppression of the neurons' responses to sugars, we applied sucrose solution laced with IMD, TMX and CLO in an ascending series of concentrations from 1 nM to 1 μM (Fig. 2g, h). None of the concentrations we tested altered the spiking activity of sucrose-sensitive gustatory neurons in the bumblebees' or the honeybees' sensilla (Fig. 2g, h, Extended Data Table 5). (Note: we confirmed that the mean spike rates reported in Fig. 2h were not a result of simultaneous excitation of bitter neurons and inhibition of sucrose-sensing neurons by manually spike sorting the records for IMD, Extended Data Fig. 4.) Furthermore, we found that both forager and newly emerged honeybees lack taste neurons that respond to these compounds (Extended Data Fig. 3b). Therefore, the behavioural data and electrophysiological recordings from mouthparts' gustatory neurons lead us to conclude that bumblebees and honeybees cannot taste neonicotinoids in nectar.

The preference of the bees in our assays for solutions containing IMD or TMX probably arises from the pharmacological action of these compounds on nicotinic acetylcholine receptors (nAChRs) in the bees' brains. It does not reflect a generalized enhancement of feeding

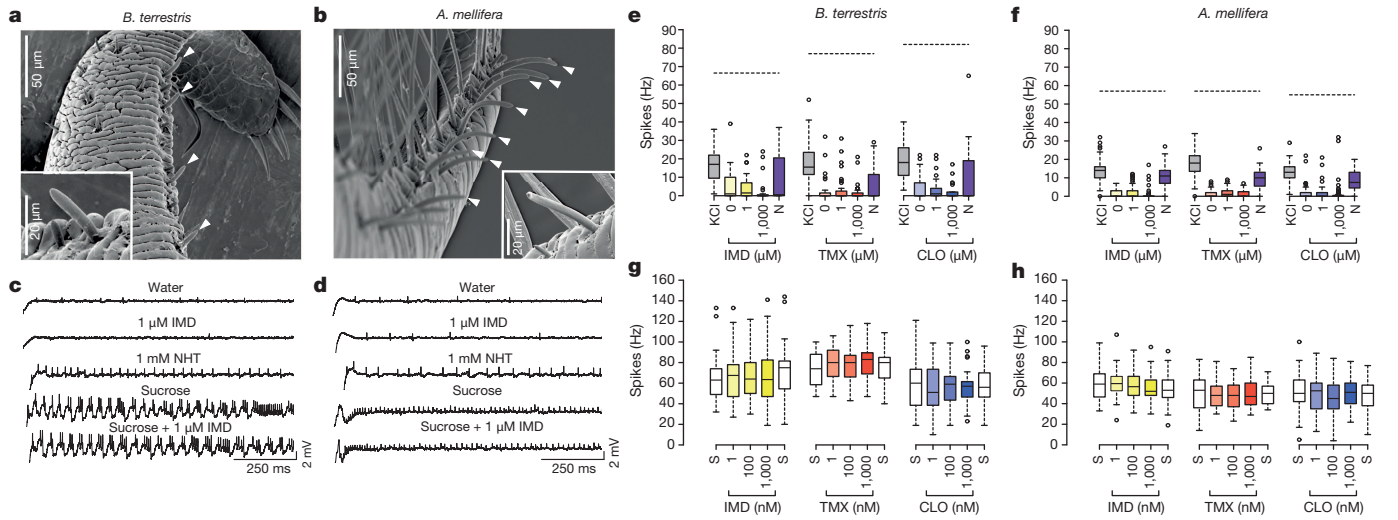

Figure 2 | Electrophysiological recordings of the gustatory receptor neurons from the mouthparts of bumblebees and honeybees during stimulation with neonicotinoids. **a**, **b**, Scanning electron micrographs (SEM) of the galea of bumblebees (**a**) and honeybees (**b**). Recordings were made from the basiconic sensilla of the galea (white arrows); inserts are higher resolution SEM of individual sensilla. **c**, **d**, Spike trains recorded from both species reveal responses to NHT and to sucrose, but not to IMD. **e**, **f**, Boxplots of the spiking responses of gustatory neurons of the mouthparts of bumblebees (**e**) and honeybees (**f**) to KCl, NHT and two concentrations of each of the neonicotinoids. Dashed lines represent the median response to 50 mM sucrose. Solutions of the three neonicotinoids did not elicit activity from gustatory neurons greater than the response to water (indicated as '0' on *x* axis) (Extended Data Table 5, ANOVA: bumblebees: $F_{2,77} = 0.935$, $P = 0.397$; honeybees: $F_{2,144} = 2.38$, $P = 0.096$). (Note: NHT elicited spike frequencies in gustatory neurons greater than those elicited by water in only 11/17 of the bumblebees we tested, whereas NHT elicited spike frequencies greater than water in all of the honeybees tested). Sample sizes: bumblebees: $n_{IMD} = 5$; $n_{TMX} = 7$; $n_{CLO} = 5$. Honeybees: $n_{IMD} = 5$; $n_{TMX} = 5$; $n_{CLO} = 6$. **g**, **h**, The spiking response to sucrose was not reduced by the presence of the neonicotinoids at concentrations in the nectar-relevant range (Extended Data Table 5, ANOVA: bumblebees: $F_{1,86} = 0.579$, $P = 0.449$; honeybees: $F_{1,127} = 2.00$, $P = 0.053$). Bumblebees: $n_{IMD} = 8$; $n_{TMX} = 5$; $n_{CLO} = 6$. Honeybees: $n_{IMD} = 6$; $n_{TMX} = 5$; $n_{CLO} = 6$. Boxplots represent the median (black bars), the 1.5 interquartile range (whiskers) and outliers (circles). Stimuli on *x* axes of **e**–**h** are in order of presentation during the experiment. Bumblebees in both experiments were randomly selected from 8 colonies; honeybees in both experiments were randomly selected from 4 colonies. N, NHT; S, sucrose.

because bees consuming these pesticides ate less food overall. Remarkably, the preference occurred even when bees consuming these solutions were more likely to die. Our data may indicate, therefore, that IMD and TMX affect the neural mechanisms involved in learning about the location of rewarding food. Previous studies have demonstrated that free-flying honeybees prefer to collect sucrose solutions containing low concentrations of nicotine[24]. Nicotine also activates nAChRs[25] expressed throughout the bee brain, including the mushroom bodies required for learning and memory[26,27]. It is notable that several studies have shown that chronic neonicotinoid administration impairs olfactory learning and memory in honeybees[1,8,28,29]. Our finding that bees acquire a preference for food laced with IMD or TMX could be explained by shorter neonicotinoid exposure in our experiments or by differential sensitivity of the nAChRs in the relevant brain regions necessary for each task[26]. It is also plausible that differential sensitivity of nAChRs accounts for our observed avoidance of newly emerged bees towards solutions containing IMD.

Consumption of neonicotinoid-laced nectar by foraging bees could lead to higher attrition in this behavioural caste as well as reducing their foraging efficiency for pollen[2,30]. This would have a greater impact on solitary bee species and on wild bee colonies with relatively few foragers than on honeybee colonies. If foragers prefer to collect nectar containing IMD and TMX, they will also bring more neonicotinoid-laced food back to the colony. For these reasons, whole colonies could be exposed to higher levels of these pesticides in the field than had been predicted previously. Mitigation strategies that rely on planting alternative sources of nectar and pollen, therefore, might not be enough to decrease the risk of poisoning pollinators with pesticides. Instead, long-term changes to policy that include reducing their use may be the only certain means of halting pollinator population decline.

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.

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Author Contributions S.C.K. performed the ephys experiments, spike-sorted the ephys data and wrote portions of the manuscript, E.J.T., K.L.S., S.D., J.M. and S.S. performed the choice experiments, E.J.T. and J.C.S. wrote portions of and edited the manuscript, and G.A.W. designed the experiments, analysed all data, and wrote the manuscript.

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 G.A.W. (jeri.wright@ncl.ac.uk).

METHODS

Behavioural two-choice assays. Experiments were performed at Trinity College, Dublin with *Bombus terrestris dalmatinus* (Unichem Ltd, Co. Dublin, Irish distributor for Koppert). Colonies were maintained at 25–30 °C in 24 h darkness and fed commercial pollen and Biogluc (Agralan Ltd, Swindon) bee food *ad libitum*. Experiments were also performed at Newcastle University, Newcastle upon Tyne with *Bombus terrestris audax* (Biobest, Belgium) and *Bombus terrestris terrestris* (Koppert Biological Systems, NATURPOL, Netherlands). Bees from 3–5 different colonies were used for each neonicotinoid. Individual worker bumblebees were collected as they tried to exit the colony. For the experiments with newly emerged bumblebees, colonies were monitored for newly emerged bees daily; newly emerged adults were identified by their pale colour. These bees were extracted using forceps from within the colony. As previously described in Tiedeken *et al.* (2014)[16], individual bumblebees were cold anaesthetized, weighed and sex-determined, and transferred to individual 650 ml plastic containers (160 × 110 × 45 mm). Containers were fitted with three 3 ml feeding tubes, inserted horizontally. Feeding tubes had four 2 mm holes so bees could alight on the tubes and feed from the openings. The feeding tubes contained one of three solutions: (1) deionized water; (1) 0.5 M sucrose; or (3) 0.5 M sucrose with a specific concentration of a neonicotinoid compound. Whether or not the bee was alive was noted 24 h after the start of the experiment. Bees that did not drink from either tube were excluded from the final analysis; the total number of these subjects was never greater than 3 per treatment (note: these subjects were always dead and likely to have died from stress or other causes).

Experiments with honeybees (*Apis mellifera* var. Buckfast) were performed at Newcastle University during the summer months using 2 free-flying outdoor colonies originally obtained from the UK's National Bee Unit (Sand Hutton, Yorkshire). Foraging adult worker honeybees were collected at the colony entrance as they returned from foraging; newly emerged adult workers were collected from brood comb as they emerged in a purpose-built box kept in an incubator at 34 °C. Bees were cold anaesthetized before placing in rearing boxes. Cohorts of 25 bees were placed in rearing boxes as previously described in Paoli *et al.* (2014)[31]. Five food tubes (as described above) were provided: (1) one with deionized water; (2) two with 1 M sucrose; (3) two with 1 M sucrose containing a specific concentration of a neonicotinoid. The number of bees alive in each cohort was counted at the time of measurement of the food consumption (24 h later).

All of the two-choice experiments were performed experimenter-blind (except IMD with bumblebees). Three neonicotinoid pesticides, imidacloprid (IMD), thiamethoxam (TMX) and clothianidin (CLO), were used in the experiments (Pestanal, Sigma-Aldrich). The neonicotinoid concentrations used were 1 nM, 10 nM, 100 nM, 1 μM (see Extended Data Table 4 for conversions to ppb and ng per bee). Bees were kept in continuous darkness for 24 h at constant temperature and 60% RH (bumblebees: 28 °C; honeybees: 34 °C). Control boxes identical to the experimental boxes (without bees) for each neonicotinoid treatment were placed in the incubator simultaneously with the experiments to measure the rate of evaporation from the food solutions. Feeding tubes were weighed, placed in the experimental boxes with the bees for 24 h, and then removed and weighed a second time. The position of the treatment tubes was randomized across subjects. The amount of solution consumed was determined as the difference in the weight of each tube after 24 h; the average value for the evaporation control for each treatment was subtracted from this final value for each tube. For bumblebees, sample sizes were: IMD: 1 nM = 57, 10 nM = 66, 100 nM = 65, 1 μM = 66; TMX: 1 nM = 38, 10 nM = 39, 100 nM = 36, 1 μM = 40; CLO: 1 nM = 57, 10 nM = 59, 100 nM = 48, 1 μM = 62. For honeybees, $n = 40$ cohorts of 25 bees per treatment. Sample size was chosen as $n \geq 40$ based on previous work[16]; sample size varied because some individuals died from unknown causes at the start of the experiments. No statistical methods were used to predetermine sample size.

Honeybee antennal and mouthparts assays. Honeybees were collected at the entrance of an outdoor colony as they returned from foraging, cold-anaesthetized, and harnessed as described in Bitterman *et al.* (1983)[32]. Each was fed 1 M sucrose to satiety and left overnight in a humidified plastic box and assayed ~ 18 h later. Briefly, two assays were employed: one in which individual honeybees were lightly tapped on the antenna with a stimulating solution (for example, sucrose) to elicit the feeding reflex (that is, proboscis extension reflex, or PER) and a second assay in which a droplet of stimulating solution was placed at the end of the extended proboscis to test whether bees would consume it (further details described in Wright *et al.* 2010[17]). Stimulating solutions were 1 M sucrose containing one of the following concentrations (1 nM, 10 nM, 100 nM, 1 μM, 10 μM) of one of three neonicotinoids (IMD, TMX, CLO).

Electrophysiology. Individual bumblebees (*B. terrestris audax* and *B. terrestris terrestris*) and honeybees were cold-anaesthetized on ice for 3–5 min, and then restrained in a metallic restraining harness as described in Bitterman *et al.* (1983)[32]. To avoid any movements of the mouthparts during recordings, muscles that trigger proboscis retraction were cut by making an incision at the level of the proboscis fossa. Each galea was fixed with a curved metallic wire pinned into dental wax.

Electrophysiological recordings were made from taste neurons located in the first 11 sensilla chaetica[33] located at the tip of the galea on the honeybee's proboscis as in Wright *et al.* (2010)[17] and in the first 6 sensilla in bumblebees. Bees were electrically grounded via a chlorinated silver wire inserted into the head. Sensilla were visualized under a microscope (M205C, Leica, Germany) at a magnification of ×256. To record from gustatory neurons, we used a method first described by Hodgson *et al.* (1955)[34]. Sensilla were stimulated with a recording borosilicate electrode (50 mm long, 20 μm diameter) containing the test compounds diluted in demineralized water. The recording electrode was connected via a chlorinated sliver wire to a high impedance 'non-blocking' pre-amplifier (TastePROBE, Syntech, Germany)[35] mounted on a motorized micromanipulator (MPC-200, Sutter Instrument, USA). The signal was further amplified and filtered with an AC amplifier (model 1800, gain: 100×, band-pass filter: 10–1,000 Hz, A-M Systems, USA). Each stimulus trial was digitized (sampling rate 10 kHz, 16 bits; DT9803 Data Translation), stored on a computer with dbWave software (version 4.2014.3.22) and analysed with Matlab R2012b (version 8.0.0.783) using PeakFinder with fixed thresholds as the peak detection algorithm (PeakFinder.m., Mathworks file ID: 25500). Recordings were made for 2 s, but only data for the first second were included in the analysis. The first 100 ms were removed to avoid the contact artefact. For bumblebees, 2–6 sensilla were sampled per bee; for honeybees, 6–10 sensilla were sampled per bee.

Recording started when the open end of the electrode was placed over the tip of the sensillum. Individuals were repeatedly sampled in one of two protocols: (1) 50 mM sucrose, 100 mM KCl, water, 1 μM neonicotinoid, 1 mM neonicotinoid, 1 mM NHT, 100 mM KCl, 50 mM sucrose; or (2) 50 mM sucrose, 50 mM sucrose + neonicotinoid in one of the following concentrations (1 nM, 10 nM, 1 μM), 50 mM sucrose. The neonicotinoids IMD, TMX, or CLO were used in each protocol. Neonicotinoid (Pestanal, Sigma-Aldrich) solutions were prepared as serial dilutions starting with 1 mM concentration. Sucrose and nicotine tartrate were purchased from Sigma-Aldrich and KCl from Fisher Scientific at purity ≥ 98%. Demineralized water was used to prepare all solutions. Intervals between stimuli were 2–5 min.

Recordings with IMD diluted in sucrose (Extended Data Fig. 4) were further analysed using dbWave (http://perso.numericable.fr/frederic.marion-poll/deterrents/tk/dbwave/index.htm). Predicted spiking neurons or 'units' were sorted from the digitally filtered signals according to their amplitude with the help of interactive software procedures. Electrophysiological recordings were then visually inspected to search for spike doublets, that is, two spikes separated by an interspike interval shorter than the silent period[36,37]. Spike trains were analysed over 1 s following the first 100 ms removed to avoid the contact artefact.

Electron microscopy. Scanning electron microscopy was performed using a Cambridge Stereoscan 240 on samples that had been fixed with glutaraldehyde, washed in phosphate buffer then dehydrated through an ethanol gradient followed by critical point drying. Specimens were then mounted on an aluminium stub with Acheson's silver dag before gold coating with a Polaron SEM coating unit.

Statistics. All analyses were performed using IBM SPSS v 19. The mean total number of spikes in the electrophysiological recordings was analysed using repeated-measures analysis of variance (ANOVA) for each species with neonicotinoid as a main effect, sensillum number and bee as covariates, and stimulus as a repeated measure; a Levene's test was employed to test for equality of variance. Post hoc comparisons were pairwise *t*-tests with a Bonferroni adjustment for experiment-wise error rate. A two-way generalized linear model (GLM) was used to compare the behaviour of bees fed each of the neonicotinoid treatments for each bee species with least squares post hoc comparisons (Note: the sucrose-sucrose choice data were not included because of the requirements of GLM for factorial design). The difference in the amount eaten between the 2 food tubes in the behavioural choice assays was also analysed using a one-sample *t*-test against zero for each treatment; critical values were Bonferroni-adjusted. The proportion of bees alive after 24 h was analysed using logistic regression (lreg). Each individual bee was entered in the analysis for the experiments with bumblebees and with honeybees. For the analysis with honeybees, 'cohort' was entered as a covariate. No statistical methods were used to predetermine sample size.

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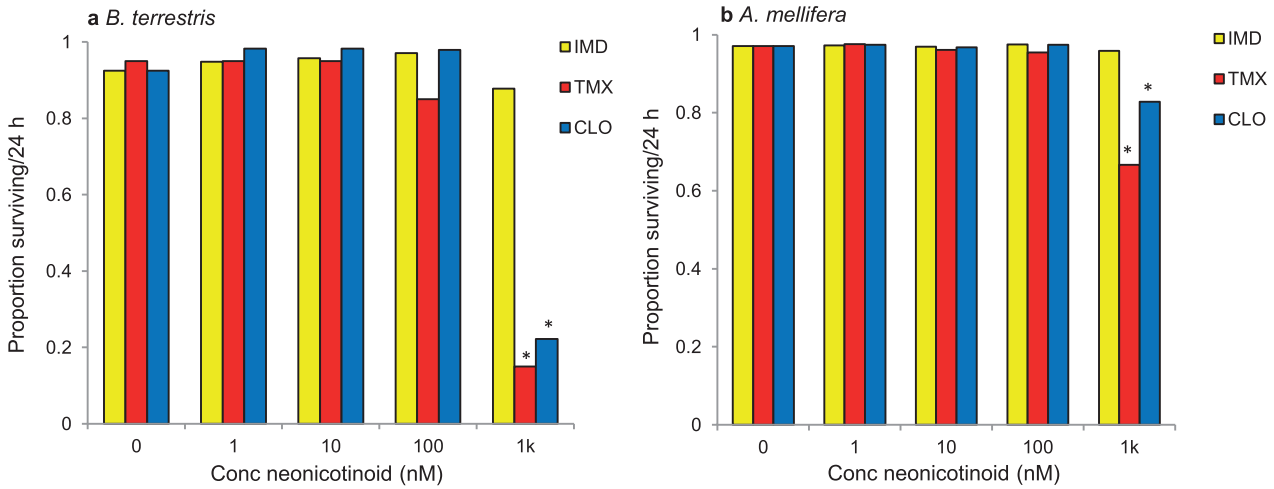
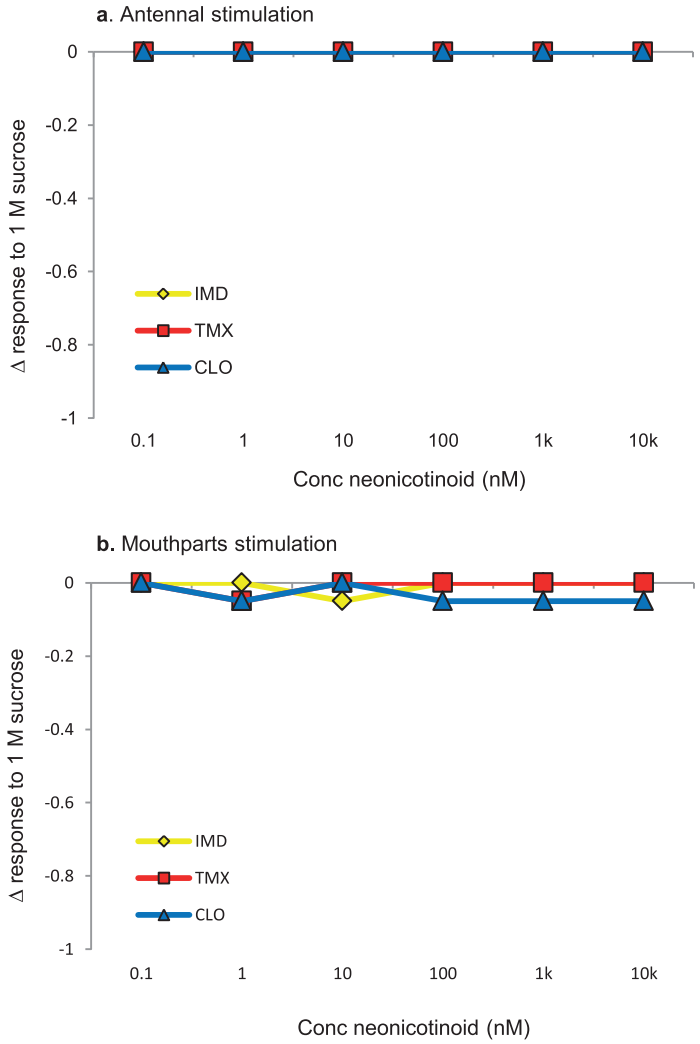
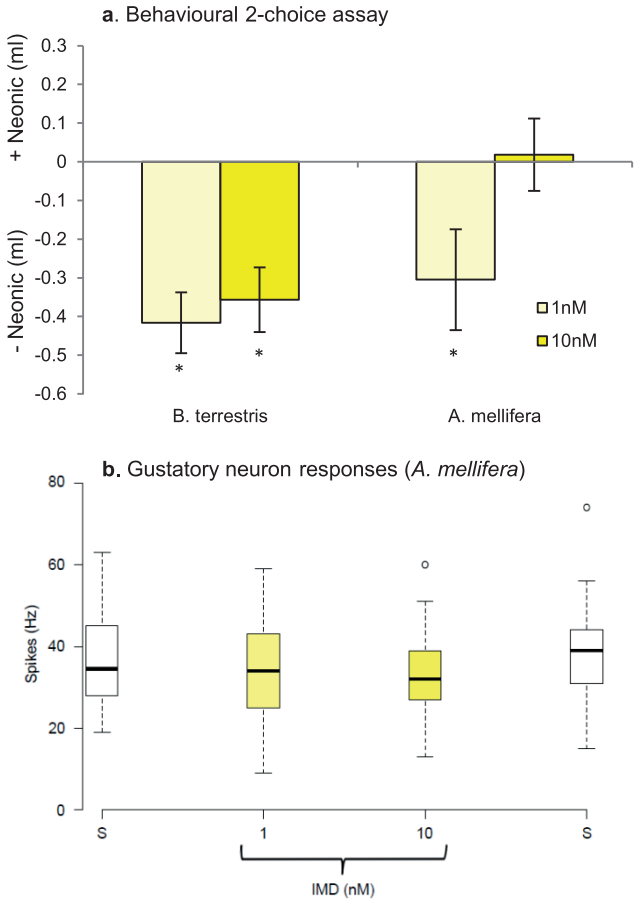


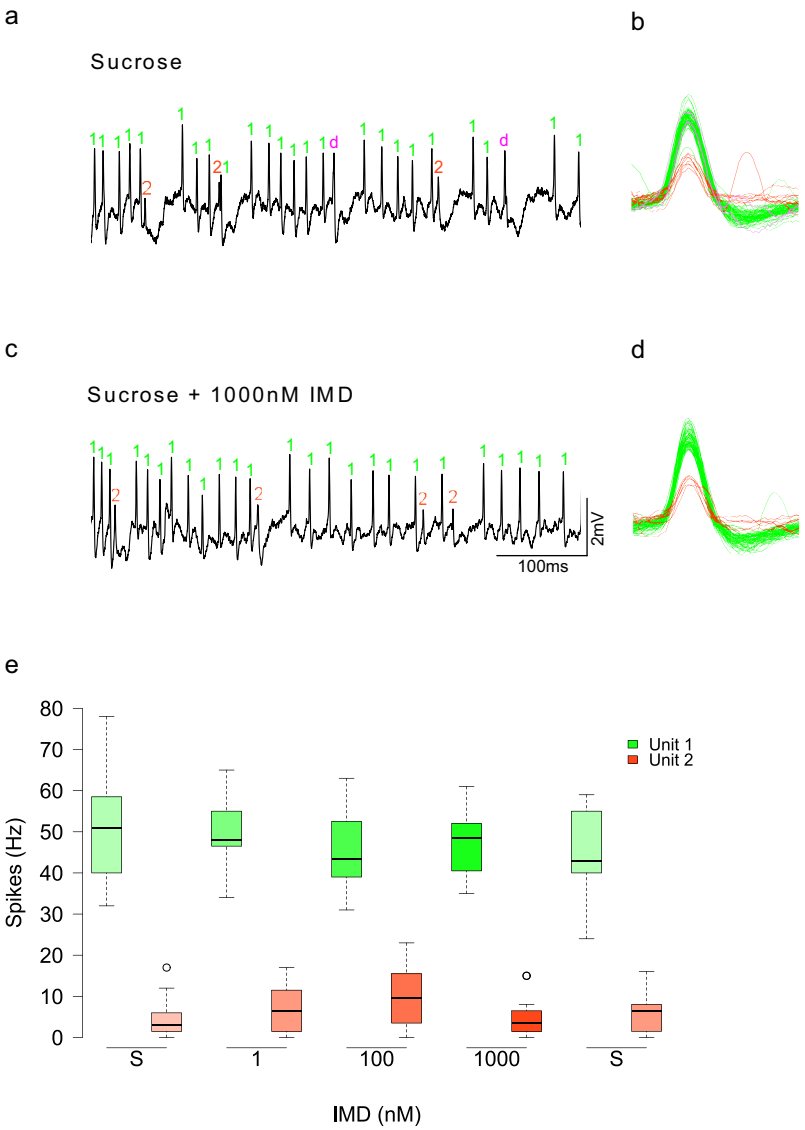
Extended Data Figure 1 | The proportion of bees surviving after 24 h in the two-choice assay. Data from Fig. 1. **a**, Bumblebees given a choice between sucrose and sucrose laced with 1,000 nM TMX or CLO were less likely to survive after 24 h (lreg: IMD: $\chi_4^2 = 4.36$, $P = 0.359$; TMX: $\chi_4^2 = 62.3$, $P < 0.001$; CLO: $\chi_4^2 = 79.7$, $P < 0.001$). **b**, Honeybees given a choice between sucrose and sucrose laced with 1,000 nM TMX or CLO were less likely to survive after 24 h (lreg: IMD: $\chi_4^2 = 5.18$, $P = 0.269$; TMX: $\chi_4^2 = 577$, $P < 0.001$; CLO: $\chi_4^2 = 243$, $P < 0.001$). Cohort (cov) accounted for a significant portion of the variance in survival for all three treatment groups (lreg: IMD: $\chi_1^2 = 22.0$, $P < 0.001$; TMX: $\chi_1^2 = 32.4$, $P < 0.001$; CLO: $\chi_1^2 = 70.2$, $P < 0.001$). Sample sizes are the same as in Fig. 1. $^*P < 0.05$ in least squares post hoc comparisons against sucrose in each treatment



Extended Data Figure 2 | Antennal proboscis extension response (PER) and mouthparts assay of honeybees to solutions containing neonicotinoids. **a**, Stimulation of the antennae with 1 M sucrose solutions containing neonicotinoids did not affect the elicitation of PER. **b**, Honeybees did not refuse to consume solutions containing neonicotinoids; only one bee in the CLO treatments failed to drink the solutions. $n = 40$ per neonicotinoid treatment for antennal stimuli and $n = 10$ for each concentration of each neonicotinoid for the mouthparts taste assay. Bees were randomly selected from 2 colonies.



Extended Data Figure 3 | Young bees avoid solutions containing neonicotinoids. **a**, Newly emerged worker bumblebees ($n = 30$ bees per treatment) and honeybees ($n = 20$ boxes per treatment) were tested in the behavioural choice assay with 1 nM and 10 nM IMD in sucrose solution as in Fig. 1. Bumblebees avoided consuming both solutions containing IMD (one-sample t-test against 0, 1 nM: $P < 0.001$, 10 nM: $P = 0.001$), whereas honeybees avoided only the 1 nM concentration (one-sample t-test against 0, 1 nM: $P = 0.003$, 10 nM: $P = 0.773$). Error bars represent ± s.e.m. **b**, The presence of IMD did not alter the spike frequency of gustatory neurons in the galeal sensilla of newly emerged honeybees (repeated-measures ANOVA, stimulus: $F_{1,47} = 0.207$, $P = 0.653$). Recordings were made from the basiconic sensilla on the galea as in Fig. 2. Boxplots represent the frequencies of responses to 50 mM sucrose or to 50 mM sucrose solutions containing 1 nM or 10 nM IMD. $n = 5$ bees, 10 sensilla per bee. Boxplots represent the median (black bars), the 1.5 interquartile range (whiskers) and outliers (circles). Stimuli on x axis are in order of presentation during the experiment.



Extended Data Figure 4 | Spike-sorted recordings. Data from four of the honeybees in Fig. 2h. **a**, To verify that the spike rates we observed in Fig. 2h were not a result in changes in the rates of firing of individual neurons, we spike-sorted recordings from four honeybees stimulated with sucrose and IMD. **b**, Spike sorting revealed two potential spiking neurons (units) characterized by different spike amplitudes; both units spiked in response to sucrose stimulation. (This was also observed previously by Wright *et al.* 2010[17]). One neuron is labelled in green, the other in red. Spike doublets (indicated in pink as 'd') where both neurons spiked nearly simultaneously were also observed. **c**, **d**, These same two spiking neurons continued to respond when stimulated with sucrose containing 1 μM IMD. **e**, Boxplots reveal that the rate of spiking was lower on average for one of the neurons (repeated-measures ANOVA, unit: $F_{1,36} = 596$, $P < 0.001$). The rate of firing of both neurons was not affected by IMD concentration (repeated-measures ANOVA, unit: $F_{1,36} = 0.369$, $P = 0.547$). Spikes from additional neurons (units) were not detected, and so we concluded that no other neurons were recruited during stimulation with IMD. 'S' indicates stimulation with sucrose. Boxplots represent the median (black bars), the 1.5 interquartile range (whiskers) and outliers (circles). Stimuli on *x* axis are in order of presentation during the experiment.

Extended Data Table 1 | Concentrations of neonicotinoids reported in floral nectar

Source	Imidacloprid			Thiamethoxam			Clothianidin		
	ng/g	PPB	nM	ng/g	PPB	nM	ng/g	PPB	nM
Schmuck et al. 2001[7]	1.9	1.9	7.43	-	-	-	-	-	-
Pohorecka et al. 2012[38]	0.6	0.6	2.34	4.2	4.2	14	2.3	2.3	9.2
Dively and Kamel 2012[6]	0.4-11	0.4-11	1.5-43	8.2-9.5	8.2-9.5	28-37	-	-	-
Stoner and Eitzer 2012[39]	10	10	39	11	11	37	-	-	-
Byrne et al. 2013[40]	2.9-39	2.9-39	11-154	-	-	-	-	-	-
Larson et al. 2013[41]	-	-	-	-	-	-	171	171	684
Pilling et al. 2013[42]	-	-	-	0.65-2.4	0.65-2.4	2.2-8.2	-	-	-
Defra 2013[43]	0.13	-	0.5	1-3.9	1-3.9	3.4-13	0.18-4	0.18-4	0.7-16

References 38–43 are cited in this table.

Extended Data Table 2 | Generalized linear models for the neonicotinoid choice experiment and total food consumption

B. terrestris	**Choice test**			**Total food consumption**		
Between-subjects contrasts	**df**	**χ^2**	**P-value**	**df**	**χ^2**	**P-value**
Concentration	3	27.9	**<0.001**	3	263	<0.001
Neonicotinoid	2	12.1	**0.002**	2	150	<0.001
Neonic x Conc	6	7.97	0.240	6	47.7	**<0.001**
A. mellifera	**Choice test**			**Total food consumption**		
Between-subjects contrasts	**df**	**χ^2**	**P-value**	**df**	**χ^2**	**P-value**
Concentration	3	4.93	0.176	3	37.1	**<0.001**
Neonicotinoid	2	11.1	**0.004**	2	10.5	**0.005**
Neonic x Conc	6	5.89	0.435	6	11.4	0.076

Data from Fig. 1. Values in bold indicate interpreted model parameters. Note: sucrose–sucrose (control) data were not included.

Extended Data Table 3 | One-sample *t*-tests against '0' for each treatment of the 24 h behavioural assay

B. terrestris									
		IMD			TMX			CLO	
	N	t(df)	P-value	N	t(df)	P-value	N	t(df)	P-value
Sucrose	55	-0.24(54)	0.402						
1nM	57	5.13(56)	**<0.001***	38	3.11(38)	**0.002***	57	0.22(56)	0.246
10nM	66	2.39(65)	**0.010**	39	3.11(37)	**0.002***	59	0.26(58)	0.183
100nM	65	2.33(64)	**0.012**	36	1.31(35)	0.099	48	0.09(47)	0.465
1µM	66	-2.6(65)	**0.005**	40	-1.15(39)	0.128	62	-2.36(61)	**0.021**
A. mellifera									
		IMD			TMX			CLO	
	N	t(df)	P-value	N	t(df)	P-value	N	t(df)	P-value
Sucrose	40	-0.85(39)	0.199						
1nM	40	1.93(39)	**0.031**	40	-0.32(39)	0.376	40	-0.288	0.387
10nM	40	1.75(39)	**0.044**	40	3.80(39)	**<0.001***	40	0.882	0.191
100nM	40	2.97(39)	**0.002***	40	3.23(39)	**0.001***	40	-0.221	0.414
1µM	40	2.00(39)	**0.026**	40	3.25(39)	**0.001***	40	0.423	0.337

Data from Fig. 1. *P* values are for 1-tailed tests. *P* values in bold are below $P = 0.05$. *Application of a Bonferroni adjustment criterion alters the *P* value threshold from $P = 0.05$ to $P = 0.002$.

Extended Data Table 4 | Comparison of doses consumed by each bee species for each treatment

B. terrestris												
	1nM			10 nM			100 nM			1μM		
	ml/bee	PPB	ng/bee/24 h	ml/bee	PPB	ng/bee/24 h	ml/bee	PPB	ng/bee/24 h	ml/bee	PPB	ng/bee/24 h
IMD	0.257	0.256	0.064(0.043)	0.167	2.56	0.418(0.337)	0.159	25.6	3.98(3.22)	0.055	256	13.9(18.4)
TMX	0.360	0.292	0.105(0.077)	0.357	2.92	1.05(0.862)	0.354	29.2	10.3(8.74)	0.115	292	33.6(33.9)
CLO	0.279	0.250	0.070(0.065)	0.259	2.50	0.647(0.600)	0.211	25.0	5.28(4.93)	0.041	250	10.3(13.6)
A. mellifera												
	1nM			10 nM			100 nM			1μM		
	ml/bee	PPB	ng/bee/24 h	ml/bee	PPB	ng/bee/24 h	ml/bee	PPB	ng/bee/24 h	ml/bee	PPB	ng/bee/24 h
IMD	0.046	0.256	0.012(0.010)	0.046	2.56	0.118(0.103)	0.045	25.6	1.16(0.974)	0.045	256	11.7(9.95)
TMX	0.040	0.292	0.012(0.011)	0.048	2.92	0.141(0.117)	0.036	29.2	1.07(1.02)	0.035	292	10.3(8.63)
CLO	0.043	0.250	0.011(0.010)	0.044	2.50	0.112(0.101)	0.043	25.0	1.08(0.868)	0.034	250	8.51(7.86)

Data from Fig. 1. Note: ng/bee values were calculated based on the mean values consumed from the neonicotinoid-containing food tubes for each treatment (ml/bee). This calculation is the product of the ng/μl of neonicotinoid in the food solution and the amount of solution eaten (μl) per bee in 24 h. The values in parentheses in the ng/bee/24 h column are the expected values if bees had eaten from both tubes equally. This value was calculated by dividing the total amount eaten for each treatment in Fig. 1c and d by 2 and using this quantity to estimate the dose.

Extended Data Table 5 | Repeated-measures ANOVA

B. terrestris	**Water**			**Sucrose solution**		
Within subjects contrasts	**df**	**F**	**P-value**	**df**	**F**	**P-value**
Stimulus	1	8.60	0.004	1	0.579	0.449
Stimulus x bee (cov)	1	4.45	0.038	1	1.23	0.271
Stimulus x sensillum (cov)	1	0.038	0.846	1	0.558	0.458
Stimulus x neonicotinoid	2	0.935	0.397	2	0.287	0.752
Error(stim)	77			86		
Between subjects contrasts	**df**	**F**	**P-value**	**df**	**F**	**P-value**
Neonicotinoid	2	10.2	0.937	2	0.004	0.996
Bee (cov)	1	0.164	0.686	1	0.871	0.354
Sensillum (cov)	1	5.63	0.020	1	3.35	0.071
Error	77			86		
A. mellifera	**Water**			**Sucrose solution**		
Within subjects contrasts	**df**	**F**	**P-value**	**df**	**F**	**P-value**
Stimulus	1	95.6	<0.001	1	7.47	0.007
Stimulus x bee (cov)	1	4.20	0.042	1	5.31	0.023
Stimulus x sensillum (cov)	1	0.303	0.583	1	0.142	0.707
Stimulus x neonicotinoid	2	2.38	0.096	2	3.00	0.053
Error(stim)	144			127		
Between subjects contrasts	**df**	**F**	**P-value**	**df**	**F**	**P-value**
Neonicotinoid	2	1.23	0.295	2	6.70	0.002
Bee (cov)	1	0.335	0.563	1	1.67	0.198
Sensillum (cov)	1	1.37	0.244	1	12.6	0.001
Error	144			127		

Data from Fig. 2. Note: for 'Water' model, the stimulus variable included: sucrose, KCl, nicotine, water, 1 μM, and 1 mM neonicotinoid. For the 'sucrose solution' model, the stimulus variable included: sucrose, 1 nM, 100 nM, and 1 μM neonicotinoid. The significant 'stimulus × neonicotinoid' term in the sucrose solution experiment for honeybees reflects a slight adaptive effect that occurred in the experiments with IMD, but not with TMX or CLO. Pairwise comparisons of each stimulus applied in the IMD experiment revealed that the 1 μM IMD and the final sucrose control stimulus produced fewer spikes than the first sucrose stimulus ($P = 0.024$ and $P = 0.002$). However, the 1 μM IMD and the final sucrose stimulus were not significantly different ($P = 0.546$) indicating either that the neurons in these experiments exhibited a slight adaptation effect or that the 1 μM IMD concentration had a toxic effect that influenced the integrity of their responses to sucrose.