Mouthparts of the bumblebee (*Bombus terrestris)* exhibit poor acuity for the detection of pesticides in nectar

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Author contributions: G.A.W, J.S, and R.H.P designed research. J.S, A.L.D, M.H, and A.E.MD-R collected behavioural data. R.H.P and H.J. collected electrophysiology data, R.H.P performed statistical analyses and prepared visualizations. R.H.P, J.S, A.L.D., and G.A.W. wrote the manuscript.

Abstract (max 200 words)

Bees are important pollinators of agricultural crops, but their populations are at risk when pesticides are used. One of the largest risks bees face is poisoning of floral nectar and pollen by insecticides. Studies of bee detection of neonicotinoids have reported contradictory evidence about whether bees can taste these pesticides in sucrose solutions and hence avoid them. Here, we use an assay for the detection of food aversion combined with single-sensillum electrophysiology to test whether the mouthparts of the buff-tailed bumblebee (*Bombus terrestris*) detect the presence of pesticides in a solution that mimicked the nectar of oilseed rape (*Brassica napus*). Bees did not avoid consuming solutions containing concentrations of imidacloprid, thiamethoxam, clothianidin, or sulfoxaflor spanning six orders of magnitude, even when these solutions contained lethal doses. Only extremely high concentrations of the pesticides altered spiking in gustatory neurons through a slight reduction in firing rate or change in the rate of adaptation. These data provide strong evidence that bumblebees cannot detect or avoid field-relevant concentrations of pesticides using information from their mouthparts. As bees rarely contact floral nectar with other body parts, we predict that they are at high risk of unwittingly consuming pesticides in the nectar of pesticide-treated crops.

Keywords: Neonicotinoids, sulfoxaflor, bumblebees, oil seed rape, taste perception

Main text

Insect pollination is a critical ecosystem service. The most economically valuable pollinators are bees (Calderone, 2012; Garibaldi et al., 2014; Huang and An, 2018), especially domesticated bees that secure crop yield and quality (Meehan et al., 2011). In agricultural ecosystems, however, insecticides are widely used to protect crop yields (Klein et al., 2007). Using insecticides impacts the health and survival of bees, contributing to the recent population declines of all bee species (Goulson et al., 2015; Potts et al., 2016; Van der Sluijs et al., 2013; Vanbergen et al., 2013). Hundreds of studies have shown that exposure to sublethal concentrations of neonicotinoid pesticides (i.e. imidacloprid, IMD, thiamethoxam, TMX, and clothianidin, CLO) in food impairs foraging behaviour, homing and orientation behaviour, and olfactory learning and memory (e.g., Gill et al., 2012; Henry et al., 2012; Parkinson et al., 2022a; Schneider et al., 2012a; Williamson et al., 2014; Wright et al., 2015). Most studies administer pesticides in sugar solutions; bees consume the solution, and then a change in behaviour or performance is recorded. If bees could detect and avoid pesticides in food, however, these substances could be used to defend crops against pests without risk to bees. Only a few have explicitly tested whether bees can taste and avoid neonicotinoid pesticides in nectar (Arce et al., 2018; Kessler et al., 2015; Muth et al., 2020), but the conclusions of these studies are contradictory. In addition, other compounds that affect cholinergic signalling in the insect nervous system, such as sulfoxaflor, have been proposed as alternatives to neonicotinoids, but whether or not these compounds can be detected by the bee's sense of taste has not previously been tested.

Insects detect non-volatile compounds (i.e. tastants) through activation of GRNs in contact chemosensilla on mouthparts, antennae, and tarsi which express receptor proteins that bind with specific types of ligands (e.g. sugars, non-nutrient 'bitter' compounds, salts, etc.) (Wright, 2016). When a taste receptor binds with its ligand, this sets off a signal transduction cascade that results in depolarization of the neuron (Dethier, 1976). For example, several types of receptors tuned to bitter substances are expressed

60 in a specialized subset of GRNs (Weiss et al., 2011). When these GRNs spike in
61 response to stimulation, feeding reflexes are inhibited (French et al., 2015). Bitter
62 compound detection is also accomplished by the inactivation of sugar-sensing GRNs
63 (French et al., 2015). For compounds which are potentially toxic, simultaneous activation
64 of GRNs that directly inhibit feeding reflexes and silencing of GRNs that activate feeding
65 make it possible to form a rapid response in reaction to contact (Wright, 2016). Thus, we
66 expected that if bees could detect neonicotinoids as bitter compounds, we would see
67 activation of bitter-sensing GRNs and/or inactivation of sugar-sensing GRNs. Previously,
68 we observed that stimulation of the mouthpart's galeal sensilla with neonicotinoids in
69 water did not elicit spikes from any GRNs, nor did we see a reduction in the rate of firing
70 of sugar-sensing GRNs in the sensilla when stimulated with a mixture of sucrose and
71 neonicotinoid compound (Kessler et al., 2015).

72 In a two choice assay, we unexpectedly found that when bees were given a choice
73 between sucrose solution and sucrose containing field-relevant doses of IMD and TMX
74 over a 24 h period, the bees choose the neonicotinoid solution (Kessler et al., 2015). This
75 could indicate that bees find neonicotinoid pesticides phagostimulatory, like sugars.
76 However, we also failed to find evidence that neonicotinoid pesticides in water elicited
77 spikes in any galeal GRNs including the nutrient/sugar sensing neurons. For this reason,
78 we concluded that bees could not taste neonicotinoids in nectar or on their own in water.
79 This conclusion, however, has been challenged by a study in free-flying *B. terrestris*
80 foragers (Arce et al., 2018). They found that free-flying bumblebees preferred specific
81 concentrations of solutions laced with TMX, but only after a period of at least 10 days of
82 feeding on the solutions (Arce et al., 2018). In this study, the location of the solutions was
83 randomized, forcing a choice at the point of feeding. These authors concluded that the
84 only way bees could solve this problem was if they used their sense of taste to identify
85 the preferred solution, arguing that further work needed to be done to identify the
86 mechanism for sensation. Subsequently, however, another study in a different species of
87 bumblebees (*B. impatiens*) using methods similar to ours reported that bees are neither
88 attracted nor deterred from consuming sugar solutions containing neonicotinoids,

consistent with the idea that they cannot detect the pesticides (Muth et al., 2020). Thus, whether or not bumblebees can taste neonicotinoids in food remains unresolved.

Bees have highly specialized mechanisms for encoding sugars which are different to those reported for any other insect species. For example, in bumblebee galeal sensilla, stimulation with sugars produces coherent spiking in 2 GRNs which burst in response to stimulation with high-value saccharide compounds (Miriyala et al., 2018; Parkinson et al., 2022b). We recently discovered that bumblebees also highly value the monosaccharide, fructose, and have a GRN tuned to detect it (Parkinson et al., 2022b). It is possible that changes to the burst pattern of firing could indicate that the presence of a compound such as a neonicotinoid in food. Furthermore, the type of sugar used to stimulate the sensilla could influence whether bees could taste neonicotinoids (Parkinson et al., 2022b). We also know that the labial palps are critical to toxin detection in other insect species (Chapman and Sword, 1993), and could house additional GRNs sensitive to bitter compounds like pesticides. Our previous work on neonicotinoid taste used sucrose to stimulate the galeal sensilla and did not analyse how pesticides might alter the burst structure of the galeal GRN response to sugars or test the labial palps. Clearly, deeper investigations could determine whether the mouthparts GRNs possess novel gustatory mechanisms for pesticide detection and provide critical information about the risk to bees of consuming the nectar of pesticide-defended crops in the field. They would also elucidate novel mechanisms for the detection of bitter compounds.

Here, we use a combination of sensitive behavioural assays and electrophysiology to test in detail whether bumblebee mouthparts have mechanisms to detect pesticides in nectar. Feeding assays using freely moving foragers make it possible to assess if bees detect and avoid potential toxins in food (Ma et al., 2016). To definitively test whether bumblebees detect neonicotinoids on their mouthparts, we quantified the structure of feeding when the mouthparts of freely-moving bumblebees (*B. terrestris)* were stimulated with nectar-like solutions containing pesticides over a 2 min period (Ma et al., 2016). We specifically tested a mixture of primarily fructose and glucose that mimicked the nectar of oilseed rape (*Brassica napus*) with field-relevant concentrations of neonicotinoids. In addition, we comprehensively tested these solutions on the gustatory receptor neurons

119 in the A-type sensilla on the galea and the labial palps of the bees' mouthparts using
120 electrophysiology to determine whether sensilla in multiple locations are able to detect
121 neonicotinoids (IMD, CLO, and TMX) and the previously untested sulfoxamine pesticide,
122 sulfoxaflor (SFX). The burst-spiking of the galeal GRNs was analysed for changes to
123 structure and compared to the GRN response to the bitter compound, quinine (QUI),
124 which has been reported previously to be a feeding deterrent to bees (Ma et al., 2016;
125 Wright et al., 2010).

126

127 **Methods**

128 *Bumblebee colonies*

129 Bumblebee colonies (*Bombus terrestris audax,* Biobest, Westerlo, Belgium*)* were
130 maintained at laboratory conditions (22-27°C and 35-40% RH) at the University of Oxford
131 and fed *ad libitum* with the proprietary sugar syrup provided with the colonies (BioGluc®,
132 Biobest, Westerlo, Belgium) containing fructose (37.5%), glucose (34.5%), sucrose
133 (25%), maltose (2%), oligosaccharides (1%), and the preservatives potassium sorbate
134 (E202) 0.15% and citric acid (E330) 0.06% (Wäckers et al., 2017). Bumblebees were
135 provided with freeze-dried honeybee collected pollen (approx. 10 g) (Agralan Growers,
136 Wiltshire, UK) three times/week.
137
138 In total, 12 colonies were used for the taste assays and 6 colonies were used for the
139 electrophysiological study. Bees were randomly assigned to a treatment and colonies
140 were counterbalanced across treatments to control for intercolony variation.

141 *Solutions*

142

143 An artificial oilseed rape nectar sugar solution was created containing 1.04 M glucose (D-
144 (+)-Glucose, Sigma Aldrich), 0.746 M fructose (D-(-)-Fructose, Sigma Aldrich), and 0.007
145 M sucrose (Sigma Aldrich) (Carruthers et al., 2017), hereafter referred to as 'OSR'.
146

147 Stock solutions (1 mM) were made for each pesticide in OSR solution. No solvents are
148 needed at these concentrations as all pesticides used are readily soluble in water at
149 concentrations far exceeding 1 mM. Concentrations of imidacloprid (IMD), thiamethoxam
150 (TMX), and clothianidin (CLO) in floral nectar range from 1.3 to 64 nM (Wood and
151 Goulson, 2017), and sulfoxaflor (SFX) can reach concentrations as high as 71.4 μM (U.S.
152 Environmental Protection Agency (EPA), 2014). In order to assess field-relevant
153 concentrations of each chemical, a concentration series of each of IMD, TMX, CLO
154 (Sigma Aldrich) and SFX (Chem Service) was made via tenfold serial dilution using OSR
155 to produce nectar containing the pesticides at 1000, 100, 10, and 1, and 0 nM (pure OSR).
156 Additionally, 0.1 mM of each chemical dissolved in OSR was assessed alongside 0.1 mM
157 and 1.0 mM QUI ((-)-Quinine hydrochloride dihydrate, Sigma Aldrich) in OSR, which
158 functioned as positive controls for the effects of a bitter tastant. A concentration of 0.1
159 mM was chosen for the pesticides as this encompasses the higher range of SFX found
160 in nectar, and allowed us to see if extremely high concentrations of IMD, TMX and CLO
161 could be detected by bees.

162 *Aversive taste assay*

163 Bumblebee workers were collected directly from the colony under red light and placed
164 individually into plastic holding vials with ventilation holes drilled into the lid. To minimise
165 inclusion of nest bees (i.e. bees that never or rarely forage), only workers with a thorax
166 width > 4.0 mm were used in these experiments (Goulson et al., 2002). In order to
167 motivate the bees to feed during the assay, bees were deprived of food between 3-6 h.
168 Bees were held in individual plastic holding vials at laboratory conditions in total darkness
169 throughout the starvation period. Following the starvation period, individual bees were
170 transferred into a 15 ml falcon tube, modified such that the tip of the tube was removed
171 and a small (10 mm x 20 mm) steel mesh was affixed contiguous with the resulting hole.
172 Bees could extend their proboscis through this hole to reach the feeding solutions, whilst
173 the mesh served as a surface for the bees to grip onto to maintain their position during
174 feeding. The tube was then affixed to a polystyrene holder with dental wax (Ma et al.,
175 2016).

176

177 A 60 mm long, 100 µL glass capillary tube was filled with a test solution and scanned onto
178 a computer at 600 dpi to produce an image from which the start volume could be
179 measured. The capillary tube was then connected to a silicone tube which was in turn
180 connected to a 1.0 ml syringe via a female connector, as in (Ma et al., 2016). This syringe
181 functioned as a pipette bulb to maintain the feeding solution at the tip of the microcapillary.
182 The capillary was held in place in the apparatus via a 1.0 ml modified syringe affixed to a
183 micromanipulator. A DinoLite digital microscope camera (Model AM4815ZT, DinoLite,
184 The Netherlands) was positioned 20 cm above the feeding site where the bees accessed
185 the test solutions. This was connected to a computer and set to record using DinoLite
186 Digital Microscope software (DinoLite, The Netherlands) at a frame rate of 640 X 480 at
187 25X magnification. All feeding behaviour was recorded for later analysis.
188

189 Once a bee was positioned in the apparatus, they were given 3 min to habituate to their
190 environment before the assay began. Following this, bees were encouraged to extend
191 their proboscis by touching the bee's terminal antennomers with a droplet (~3.5 µL) of 0.5
192 M sucrose (Sigma Aldrich) dissolved in deionised water. Once the bee extended their
193 proboscis, the droplet was presented to the bees' mouthparts for them to consume. Each
194 bee was given up to 5 min to extend their proboscis and consume the droplet. Bees that
195 did not do so were removed from the experiment. When the bee finished consuming the
196 droplet, the microcapillary tube containing the test solution was presented to the bees
197 extended mouthparts. The test phase began once the bee's proboscis contacted the test
198 solution, and the bee was given 2 min to consume the solution. After feeding,
199 microcapillaries were re-scanned to measure the end volume.

200 All experimenters were blind to the experimental treatments. All solutions were tested in
201 a randomised order and treatments were counterbalanced over time to eliminate any
202 effects of starvation time on feeding behaviour.

203 *Measuring solution consumption and feeding behaviour*

204 ImageJ (Schneider et al., 2012b) was used to produce measurements of the length of
205 solution in each microcapillary before and after the assay. The reference scale was set
206 to 60.0 mm. Image files were zoomed in to 400% and the length of the solution inside the
207 microcapillary was measured meniscus to meniscus. The length of test solution
208 consumed by each bee was calculated as the difference between the measured length
209 of the liquid inside the microcapillary tube before and after the test phase. These lengths
210 were then converted to volumes using the formula:
211
212 $$(100\ \mu L \times X\ mm) / 60.0\ mm$$
213
214 Where 100 µL is the maximum volume of the capillary tube, 60.0 mm is the length of the
215 capillary tube, and X mm is the amount of solution consumed as a measured length within
216 the capillary.
217
218 A feeding bout is defined as a period in which contact is made between the proboscis and
219 test solution that is not separated by an absence of contacts for 5 s or more (Ma et al.,
220 2016). The number of feeding bouts and their duration can be used to evaluate the
221 phagostimulatory or deterrent activity of the compounds tested (Ma et al., 2016). To
222 identify bouts of feeding behaviour, videos were played back at 50 % speed and periods
223 of contact between the bees' mouthparts and the solution were scored using the Noldus
224 Observer (Noldus, the Netherlands). The duration of the first bout and the cumulative bout
225 duration (over the 2 minute period) were compared between stimuli.

226 *Electrophysiology*

227 We performed extracellular tip recordings on individual taste sensilla on the bumblebee
228 mouthparts to assess the peripheral taste perception of sucrose, a 10% dilution of oilseed
229 rape nectar (10% OSR), and the addition of pesticides or QUI to 10% OSR. We recorded
230 from 'A-type' sensilla from the galea (Gal), which are known to produce bursting spike
231 trains in response to sugars (Miriyala et al., 2018) and the third and fourth segments of
232 the labial palps (LP_{III} and LP_{IV}, respectively, Figure 1 A). Bumblebees were prepared for
233 electrophysiology as described previously (Miriyala et al., 2018; Parkinson et al., 2022b).

234 Briefly, bees were cold-anesthetized and harnessed with the proboscis extended. The
235 mouthpart nerves were severed by making an incision at the base of the mouthparts to
236 prevent movement. The galea and labial palps were oriented for access to the sensilla
237 using dental wax and wire pins. Sensilla were stimulated for 5 s with a borosilicate (Clark
238 capillary glass 30-066, GC150TF-10) recording electrode (20 µm tip diameter, made with
239 a Narishige PC-10 electrode puller) filled with the test solution. Signals were acquired
240 with a pre-amplifier (TasteProbe; Syntech, Germany), amplified (AC amplifier 1800, A-M
241 Systems, USA), digitized at 30 kHz (DT9803 Data Translation) and stored using
242 DataView (version 11.5). Pesticides (IMD, CLO, TMX, SFX) were tested in a background
243 of 10% OSR. Pesticides were tested using a concentration gradient (1, 10, 100, and 1000
244 nM) and also at 0.1 mM. Additionally, QUI was tested in 10% OSR at concentrations of
245 0.1 and 1.0 mM. A minimum inter-stimulus interval of 3 min was used to prevent sensory
246 adaptation. We obtained recordings from n=12 bee per compound and tested 2-4 sensilla
247 per mouthpart location (6-12 sensilla per bee). To ensure the pesticides or QUI were not
248 imposing a toxic effect on the taste sensilla, we included recordings at the beginning and
249 end of each stimulation series with 10% OSR (SI Figure 1).

250 *Spike detection*

251 Spikes from GRNs on the galea were extracted as described previously (Parkinson et al.,
252 2022b). Briefly, after band-pass filtering (300-2500 Hz, second order butterworth filter)
253 and normalization, spikes were detected using the peakfinder function with adequate
254 thresholds manually set for each recording. Interspike intervals were used to detect the
255 end of burst positions (EOB). Using this method, we detected three GRNs within the
256 galeal electrophysiological recordings, as seen previously (Parkinson et al., 2022b).
257 GRNs of the labial palps were non-bursting, and visual inspection of the data suggested
258 that two GRNs were present in these recordings. The spike waveforms for each recording
259 were compared to differentiate the spikes from the putative units. Waveforms were
260 aligned from the peak +/- 2 ms, and the Matlab singular value decomposition (SVD)
261 function was used to obtain the principal components of the spike waveforms. Waveforms
262 were then projected onto the space spanned by the first two principal components, and
263 k-means clustering was used to assign the waveforms to units for each recording. Using

this method, two (or in some cases just one) GRNs were obtained from each recording, with the unit labelled GRN1 represented by larger waveform amplitudes and higher spike frequencies in response to sugars.

Statistical analyses and data presentation

We performed all further analyses in R version 4.2.1 (Team, 2016). Average firing rates of the 7 GRN types (Gal GRNs 1-3, LP_{III} GRNs 1-2, and LP_{IV} GRNs 1-2) across mouthpart locations were compared. Firing rates of a given GRN type were averaged across sensilla from a single animal, and log transformed ($\log_{10}$(average_rate + 1)) to fit a normal distribution. We compared electrophysiological responses across stimuli using linear mixed effects models (LME, lmerTest package) (Kuznetsova et al., 2017) with Gaussian distributions and "bee ID" as the random effect (avg_rate~stimulus*GRN+(1|BeeID)). Models with non-significant interaction terms were re-run without the term (avg_rate~stimulus+GRN+(1|BeeID)). *Post hoc* analyses were performed using estimated marginal means (emmeans package, (Lenth et al., 2018)) with Tukey's adjustment for family-wise error rates. Significant effects were denoted with asterisks (*, <0.05; ** <0.001; ***, <0.0001).

We also assessed the temporal patterns of GRN activity over time. Time series spike data were averaged by animal for a given GRN type, and firing rates were calculated in 0.1 s bins from 0.1 to 1.1 s. Because the labial palp GRNs did not display a bursting pattern, we averaged the firing rate within each bin across all four labial palp GRNs. We assessed the bursting pattern of galeal GRNs (Gal 1 and 2) was assessed using two measures: the burst length (number of Gal1 spikes per burst) and burst rate. We constructed firing rate histograms (firing rate versus time) using these measurements over 1 s of stimulation, and compared stimuli with LME models (temporal_parameter~stimulus+bin+(1|BeeID)) implemented in the lmerTest package (Kuznetsova et al., 2017). We denoted significant differences in the resulting slopes between stimuli on plots with letters, assessed with the estimated marginal means (emmeans) package (Lenth et al., 2018) with Tukey's adjustment for family-wise error rates. To assess differences in the complete temporal code of all 7 GRNs across mouthparts, we aligned the binned spike times in series for

294 each GRN. For each animal and stimulus, there was a vector of 70 bins (ten 0.1s bin per
295 GRN type, aligned for 7 GRNs). To reduce the variability in responses between animals
296 and balance the scale of responses across GRNs, we normalized the responses across
297 GRN replicates within each animal by dividing by the average responses of each GRN to
298 10% OSR. The normalized responses of single GRNs were then averaged across
299 replicates for each animal. We applied t-distributed stochastic neighbor embedding (t-
300 SNE) to reduce the dimensionality of the temporal data into two components for each
301 dataset (OSR vs sucrose, QUI, and pesticides), using Euclidean distance as the distance
302 metric. The results from the t-SNE were subsequently clustered to reveal the grouping of
303 responses by stimulus. To predict the optimal cluster count, we employed Monte Carlo
304 reference-based consensus clustering (M3C, (John et al., 2020)), an unsupervised
305 learning technique that aggregates across multiple k-means clustering runs. If the optimal
306 number of clusters (k) was greater than one, we included clustering on the t-SNE plots.
307
308 Measurements of feeding behaviour (volume consumed, first bout, and cumulative bout)
309 were compared using t-tests LME, with "colony" as a random effect:
310 (feeding_measurement~stimulus+ (1|ColonyID)), or
311 (feeding_measurement~stimulus+concentration+(1|ColonyID)) (lmerTest package)
312 (Kuznetsova et al., 2017), with emmeans *post hoc* tests (emmeans package) (Lenth et
313 al., 2018) and Tukey's adjustment for family-wise error rates. The non-significant
314 interaction term (stimulus*concentration) was removed for all pesticide concentration
315 gradient models due to increased model fit (using AIC and BIC). Behavioural data that
316 was not normally distributed was log transformed. For all boxplots, the median is indicated
317 by a line, bounds of the box mark the 1st and 3rd quartile, and whiskers extend to 1.5
318 times the interquartile range. Where data points are drawn, these represent the
319 responses of individual GRNs.

320 **Results**

321 ***Sugar solution composition is encoded by GRNs***

322 Responses of bumblebee galeal GRNs to individual sugar compounds have previously
323 been described (Miriyala et al., 2018; Parkinson et al., 2022b). Here, we tested whether

324 a mixture of fructose, glucose, and negligible amounts of sucrose that mimics OSR nectar
325 altered the GRN spiking responses compared to sucrose alone. We also expanded our
326 investigation to include responses form GRNs in the labial palps (Figure 1 A). We found
327 that GRNs in each location had specific patterns of activation that characterized their
328 responses to sugar stimulation (Figure 1 B). Stimulation of galeal sensilla with sucrose or
329 with OSR nectar produced bursts of spikes resulting from the co-activation of two GRNs
330 (Gal1 and Gal2), as we found for individual sugars previously (Miriyala et al., 2018;
331 Parkinson et al., 2022b, Figure 1 B). Each labial palp sensillum (LP_{III} and LP_{IV}) contained
332 two sugar-sensitive GRNs (LP_{III} 1 and 2, LP_{IV} 1 and 2), but these GRNs exhibited tonic
333 firing patterns in response to stimulation instead of bursts of spikes (Figure 1 B). The
334 spiking rates averaged over 1 s stimulation of Gal1 and the labial palp GRNs were
335 unchanged towards the two stimuli, but the average rate of Gal2 and Gal3 when
336 stimulated with sucrose was lower than the rate for stimulation with 10% OSR (Figure 1
337 C, LME, stimulus*GRN $F_{6,1607}$ = 9.16, p < 0.0001). (Note: Gal3 was active only when
338 stimulated with 10% OSR, but not by an equimolar sucrose solution, Figure 1 C).
339
340 We compared the time-varying responses of GRNs using binned spikes (0.1 s bins over
341 1 s stimulation) averaged by animal across sensilla. The coefficient of variation of spike
342 rates across sensilla for each animal for a given stimulus ranged from 0.023 to 2.44 with
343 a median of 0.35 and IQR of 0.47. We found that the rate of adaptation, measured by the
344 change in the interspike interval of labial palp GRNs were not significantly different for
345 10% OSR or sucrose (Figure 1 D, stim: $F_{1,461}$ = 3.11, p = 0.079; time, $F_{1,450}$ = 586, p <
346 0.0001; SI Figure 1 B). However, galeal GRN burst structure (Gal1 and Gal2) differed
347 between stimuli (Figure 1 E, stim*GRN: $F_{1,938}$ = 13.8, p = 0.0002, time: $F_{1,938}$ = 1.42, p =
348 0.23). OSR elicited a 20% higher burst rate (i.e., Gal2 rate, SI Figure 1 C) and shorter
349 bursts (i.e., fewer Gal1 spikes per burst, SI Figure 1 D), although the rate of adaptation
350 of Gal1 did not differ as a function of stimulus (Figure 1 E). To understand if the population
351 of neurons we recorded from contained information about stimulus identity, we combined
352 the temporal responses binned at 100 ms intervals over 1 s of recording of all 7 GRNs
353 across mouthparts using a clustering algorithm (t-SNE and k-means clustering). The

354 algorithm predicted two statistically separate stimulation groups, one for OSR and one for
355 sucrose (Figure 1 F).
356
357 In spite of the differences observed in GRN responses, the total volume of the 10% OSR
358 and equimolar sucrose solutions (1.79 M, Figure 1 G) consumed by bumblebees was not
359 significantly different in the free-feeding assay (Figure 1 H, LME, $F_{1,28} = 2.09$, $p = 0.16$).
360 We also did not observe a difference in the duration of the first feeding bout (Figure 1 I,
361 LME, $F_{1,26} = 0.176$, $p = 0.678$), or the total time the bees spent in contact with the solutions
362 over 2 min (Figure 1 J, LME, LME, $F_{1,26} = 0.555$, $p = 0.463$). We also tested whether the
363 concentration of these sugar solutions influenced behaviour using a concentration
364 gradient of OSR and sucrose solutions at 2.0, 1.5, 1.0, 0.5 M and water. A greater volume
365 of sucrose was consumed for the 1 M and 1.5 M concentrations than for the OSR solution,
366 but there was no difference at any other concentration (SI Figure 2). First bout and total
367 contact duration were not significantly different.
368
369 ***Labial palp GRNs may enable bitter detection***
370 QUI is routinely used to test an animal's sensitivity to bitter substances. Here, we tested
371 a concentration series of QUI in 10% OSR solution to test how GRNs on the labial palps
372 and galea respond to QUI, for use as a positive control for our tests with pesticides. A 1
373 mM QUI concentration suppressed all galeal and labial palp GRNs, while 0.1 mM QUI
374 suppressed the activity of $LP_{III}2$ and $LP_{IV}2$ alone (Figure 2 A, LME, stimulus*GRN $F_{12,1581}$
375 $= 11.3$, $p < 0.0001$, SI Figure 3 A-C). The rate of adaptation, however, of the labial palp
376 GRNs was faster when they were stimulated with the 0.1 mM QUI solution (Figure 2 B,
377 stim*time: $F_{1,450} = 13.8$, $p = 0.0002$). Galeal GRN burst structure was affected by the
378 addition of QUI (Figure 2 C, stim*GRN: $F_{2,1116} = 308$, $p < 0.0001$, time: $F_{1,1116} = 0.062$, p
379 $= 0.803$; SI Figure 3 B-C). Gal1 adapted faster when stimulated with 0.1 mM QUI
380 compared to QUI, although there was no effect on Gal2 (Figure 2 C). Clustering of all
381 GRN responses predicted two groups, with the responses to 1 mM QUI separating from
382 the responses to 10% OSR and 0.1 mM QUI (Figure 2 D). OSR and 0.1 mM QUI were
383 not significantly different.
384

385 The detection threshold observed in the behaviour experiments was lower than predicted
386 by the t-SNE clustering but consistent with our measurements of adaptation in labial palp
387 neurons. Bumblebees consumed significantly less OSR solution when it contained 0.1
388 mM QUI, while 1 mM QUI was completely deterrent (Figure 2 E, LME, concentration: $F_{2,35}$
389 = 49.1, $p < 0.0001$). However, the structure of feeding towards 1 mM QUI was similar to
390 the results found for the cluster analysis of the GRNs: bees tested with 1 mM QUI
391 exhibited much shorter average first feeding bout duration (Figure 2 F, LME,
392 concentration: $F_{2,40}$ = 18.2, $p < 0.0001$) and shorter average cumulative bout duration
393 (Figure 2 G, LME, concentration: $F_{2,36}$ = 61.5, $p < 0.0001$) than those fed with OSR or 0.1
394 mM QUI solution.
395
396 ***Extremely high concentrations of pesticides in OSR nectar reduce the rate of***
397 ***spiking of GRNs***
398 In our previous work (Kessler et al. (2015), field-relevent concentrations of neonicotinoid
399 pesticides were not detected by galeal GRNs. Here, we used OSR instead of sucrose
400 and included sulfoxaflor. We also extended this experiment to test the labial palps. As
401 before, we found no significant difference in galeal GRN responses between the control
402 (OSR) solution and all of the solutions containing pesticides at field-relevant
403 concentrations (SI Figure 4). No significant differences were detected in the responses of
404 the labial palp neurons to the pesticide solutions (SI Figure 4).
405
406 To test whether all concentrations of pesticides go undetected by bees, we stimulated
407 GRNs with 0.1 mM of each pesticide mixed with 10% OSR. Such concentrations are 10-
408 100k fold greater than has been reported for neonicotinoids from the nectar and pollen of
409 agricultural crops and are at least 1k fold greater than the LD_{50} values for bumblebees
410 (Cresswell et al., 2014; Mundy-Heisz et al., 2022; Siviter et al., 2022). Sulfoxaflor,
411 however, is present at much higher concentrations in the field (e.g., 71.4 μM, U.S.
412 Environmental Protection Agency (EPA), 2014)). Stimulation with extremely high
413 concentrations of TMX reduced the average firing rates of $LP_{III}2$ and $LP_{IV}2$; none of the
414 other pesticides affected the average GRN firing rates (Figure 3 A, LME, stimulus*GRN:
415 $F_{24,2139}$ = 1.65, p = 0.024.)

416

417 We also examined how the temporal response of galeal and labial palp neurons was
418 influenced by stimulation with extreme concentrations of the pesticides. Labial palp GRNs
419 adapted faster when IMD or TMX were added to 10% OSR (Figure 3 B, stim: $F_{1,821}$ =
420 49.9, $p < 0.0001$; time, $F_{1,808} = 571$, $p < 0.0001$), and firing rates of labial palp GRNs over
421 1 s were significantly reduced (SI Figure 5 A). Gal1 and Gal2 spiking and adaptation rates
422 were affected by CLO and IMD (Figure 3 C, stim*GRN: $F_{4,1653} = 3.47$, $p = 0.0078$; time,
423 $F_{1,1653} = 2.33$, $p = 0.127$). Galeal GRN bursting rate was slightly reduced when stimulated
424 with OSR mixed with CLO or IMD (SI Figure 5 B), while the burst length was not affected
425 by the addition of pesticides (SI Figure 5 C). We used the same clustering algorithm that
426 was able to differentiate the responses to sucrose and OSR. Clustering using all of the
427 data for each of the sensillum types failed to separate the pesticide responses from
428 responses to the control (10% OSR, Figure 3 D).

429

430 ***Bumblebees readily consume pesticides in nectar***

431 We assessed whether the pesticides were aversive in the range of values that included
432 field-realistic concentrations (0 - 1000 nM) and one of the extreme values (0.1mM) added
433 to a 10% OSR solution. Field-relevant concentrations of the pesticides in OSR nectar did
434 not deter the bees from feeding, as the presence of these compounds did not significantly
435 alter the amount of food consumed (LME, concentration: $F_{4,297} = 0.209$, $p = 0.933$), first
436 bout duration (LME, concentration: $F_{4,290} = 0.648$, $p = 0.629$) or cumulative bout duration
437 (LME, concentration: $F_{4,288} = 0.310$, $p = 0.871$) over the 2 min assay period (Figure 4 A-
438 C). Importantly, we also tested feeding behaviour using an extreme concentration (0.1
439 mM) of the pesticides in OSR. Even at this very high concentration, we did not observe a
440 significant difference in the amount of food consumed (LME: $F_{4,73} = 0.872$, $p = 0.485$),
441 first bout duration (LME, $F_{4,74} = 0.392$, $p = 0.814$), or cumulative bout duration (LME, $F_{4,74}$
442 $= 1.06$, $p = 0.382$) over 2 min (Figure 4 D-F).

443

Discussion and conclusions

For the first time, we report how populations of GRNs on the bee's mouthparts respond to stimulation with putatively bitter compounds. We verified that bees do not have mechanisms on their mouthparts that enable them to taste neonicotinoids or sulfoxaflor when these compounds are present in nectar. Neonicotinoids and sulfoxaflor did not change the bursting of sugar-sensing neurons in the galea, nor did they elicit or inhibit spikes in labial palp neurons. Strikingly, we show that bumblebees do not avoid drinking OSR nectar solutions even when very high concentrations of common pesticides are present. Very high concentrations of these pesticides could produce a small but measurable reduction in the spiking of sugar-sensing GRNs, but these compounds never elicited spikes on their own. What's more, these small changes in the rate of spiking did not translate into features that the clustering algorithm could use to differentiate pesticide laced stimuli from the control OSR solution. From these data, we conclude that the buff-tailed bumblebee's mouthparts do not have mechanisms for the detection and avoidance of common cholinergic pesticides in nectar.

In the present experiments, we expected that if neonicotinoids and sulfoxaflor could be tasted by bees, they would exhibit the same responses in GRNs produced by other non-nutrient compounds (i.e. bitter responses). For this reason, we were mainly testing for the reduction of GRN spiking caused by the suppression of sugar-sensing GRNs, as all of the 'bitter' compounds we tested were presented to the sensilla in OSR nectar (a mixture of primarily fructose and glucose). The known bitter compound, QUI, clearly suppressed the activity of sugar-sensing GRNs in both labial palps and the galea, but it did not measurably alter the burst pattern of firing of the galeal GRNs. Importantly, our new data were able to confirm that field-relevant concentrations of pesticides did not significantly alter the pattern or rate of spiking of galeal or labial palp GRNs towards a nectar solution.

We used a clustering algorithm that integrated input from all the sensilla we recorded from over a 1 s time interval with every stimulus to identify whether population-level input made it possible to differentiate the stimuli. Although we were able to measure small but significant reduction in the responses of both labial palp and galeal neurons caused by

475 the high concentrations (100 μM) of IMD, SFX, and TMX, this effect was not sufficient for
476 the clustering algorithm to form classes of distinct stimuli for the OSR-pesticide mixtures.
477 This is similar to what we observed for 0.1 mM QUI; though a small change in firing was
478 detected, the clustering algorithm did not differentiate it from OSR. It is worth noting that
479 the OSR background solutions in both cases were different: in the behavioural
480 experiments the concentration of OSR was an average value of that naturally found in
481 nectar. Our physiology experiments were performed using a 10% dilution of this solution.
482 Different solutions were used because it was not possible to record from neurons with
483 such a concentrated sugar solution, and in behavioural experiments, most bumblebees
484 do not find 10% OSR sufficiently phagostimulatory to feed.
485
486 Our electrophysiological recordings are the first to report GRN responses from the
487 bumblebee's labial palps. The labial palps in other insects such as locusts are used to
488 detect both phagostimulants and deterrant compounds (Blaney and Chapman, 1970), so
489 we might have rationally expected to observe labial palp GRNs that spike in response to
490 QUI and cholinergic pesticides, but we did not. This could indicate that on the mouthparts,
491 bitter compounds are only detected through the suppression of sugar-sensing GRNs (de
492 Brito Sanchez et al., 2005).
493
494 It is interesting to note that in the case of all bitter compounds tested, the output of the
495 clustering algorithm matched the behaviour of the bees, as the bees did not reject any of
496 the pesticide solutions or the 0.1 mM QUI solution after the first feeding bout. This could
497 indicate that that small differences detected at the sensory periphery do not translate into
498 a sufficient signal across the whole population of gustatory neurons to produce an
499 aversion to foods containing potential toxins. Bumblebees did drink less overall of the 0.1
500 mM QUI solution compared to OSR, however the amount of time they spent in contact
501 with the feeding solution was the same. This suggests that, while 0.1 mM QUI is not
502 immediately aversive to bumblebees (i.e., by taste), they can integrate negative post-
503 ingestive feedback within a 2 min feeding period. Compounds reach the hemolymph of
504 honeybees within 30 s of ingestion (Simcock et al., 2018), and quinine causes malaise-
505 like symptoms (Ayestaran et al., 2010; Hurst et al., 2014), so it is feasible that the

506 bumblebees in our behavioural assay factored in post-ingestive feedback while
507 consuming 0.1 mM QUI. Unfortunately, when fed high concentrations of pesticides, post-
508 ingestive feedback did not prevent bumblebees from consuming potentially lethal doses.
509 For example, the LD50 for TMX in *Bombus terrestris* is approximately 6 ng/bee (Siviter et
510 al., 2022), and bumblebees in our assay consumed a median amount of 3.5 ng of TMX
511 when dissolved at 100 uM in OSR.
512
513 Our data also indicate that gustatory coding is more than the presence or absence of
514 spikes arising from GRNs tuned to detect particular tastants. In bumblebees, it is clear
515 that the pattern of spiking itself conveys information which is a feature that enables the
516 brain to distinguish gustatory stimuli (Parkinson et al., 2022b). This supports the idea that
517 encoding of gustatory stimuli is accomplished by the whole population of GRNs on a given
518 body part (e.g. the proboscis) as was observed in multichannel recordings from the adult
519 hawkmoth maxillary nerve (Reiter et al. 2015). Thus, activity across the mouthparts GRN
520 population that includes temporal input over time is likely to be used by the brain to
521 differentiate taste stimuli and to facilitate the valuation of food quality. Our new data
522 testing the difference between a nectar stimulus composed of a mixture of sugars (OSR)
523 and sucrose clearly illustrates this. The changes in the rate of bursting and spiking in the
524 galeal and labial palps were sufficient for the clustering algorithm to classify sucrose and
525 OSR as separate stimuli. Although our behavioural assay did not show a difference, we
526 would not expect it to, as it is an assay developed to study food rejection behaviour by
527 bees, not differences in phagostimulation.

528 Previously, Arce et al. (2018) reported that *B. terrestris* were capable of selectively
529 foraging on 30% w/v sucrose solutions containing TMX. This finding confirmed our
530 previous data that honeybees and bumblebees prefer solutions containing TMX (Kessler
531 et al. 2015). Arce et al (2018) did not observe a preference for or against the solutions
532 containing the neonicotinoids during the initial days of their experiments. We expect that
533 if taste was the mechanism for the preference, the bees would have shown a preference
534 for the TMX solution within the first foraging bout, similar to the time frame of our
535 behavioural assay in this study (2 min). Instead, the authors found that the preference for

536 the TMX solution developed over time. Similarly, in our previous work, we found that
537 individual bees developed a preference for solutions containing TMX if they were confined
538 to feed on a choice between sucrose and sucrose laced with pesticide over longer time
539 period (i.e., 24 h, Kessler et al., 2015). We concluded that the preference was caused by
540 the pharmacological action of TMX on cholinergic circuits involved in encoding reward in
541 the insect brain (Barnstedt et al., 2016), as we found no electrophysiological evidence to
542 support that bees could taste neonicotinoids (Kessler et al., 2015). This conclusion is
543 consistent with the expectation that a preference resulting from post-ingestive
544 reinforcement caused by amplification of the brain's response to sugars would be
545 expected to develop following long-term, repeated exposure to a pharmacological
546 compound (Palmer et al., 2013; Wright et al., 2013). If TMX caused bees to associate a
547 higher 'reward value' with food found in a particular location, they would learn to return to
548 it in preference over other solutions. Although Arce et al. (2018) switched the feeders
549 between training and testing phases, this may not have been sufficient to rule out the
550 possibility that bees could learn the location of the food within a single feeding bout.

551 Arce et al. (2018) conducted their experiments using gravity feeders in an enclosed arena,
552 allowing bees to consume solutions *ad libitum*. Since the feeders did not have visual or
553 olfactory cues, the authors inferred that the bees were capable of tasting the TMX in the
554 solution. However, gravity feeders do not simulate how bees feed on real flowers. These
555 feeders have large openings that allow multiple bees to feed simultaneously, enabling
556 them to contact the solution with all body parts, including the tarsi and antennae. While it
557 is possible that the sensilla on the antennae or tarsi could detect TMX, it seems unlikely
558 given that honeybees have limited bitter detection in these locations, only observed when
559 the sensilla are contacted with concentrations of compounds like QUI that are >1 mM (De
560 Brito Sanchez et al., 2005; de Brito Sanchez et al., 2014). The field-relevant
561 concentrations of the pesticides we tested are between 3-6 orders of magnitude lower in
562 concentration (see Kessler et al. 2015 for review); such dilute concentrations of a bitter
563 compound present in a highly concentrated sugar solution would be difficult for most
564 organisms to detect.

565 Floral morphology such as corolla length has often co-evolved with particular groups of
566 pollinators with longer mouthparts; a long corolla protects nectar when visitors are
567 efficient and reliable pollinators. In many flowers, nectar is hidden deep within the corolla
568 such that the only contact that bumblebees have with it is via the distal end of the
569 proboscis (also the location where most of the sensilla are). For example, in OSR flowers,
570 nectaries are located at a depth of 5-8 mm (Cresswell et al., 2001) and the nectar cannot
571 be contacted otherwise unless the bee bites through the petals. In addition, bees learn to
572 handle flowers quickly to improve their foraging efficiency (Heinrich, 1976) and even
573 extend the proboscis in anticipation of entering the corolla prior to landing. Thus, they are
574 unlikely to contact nectar using gustatory sensilla on their antennae or tarsi while foraging.
575 For this reason, it is likely that nectar palatability is largely determined by the sensilla on
576 the proboscis.

577

578 **Acknowledgements**

579 We would like to thank Priya Chakrabarti-Basu for the pilot studies that led to this work.

580 **Funding**

581 This research was supported by a BBSRC grant (BB/S000402/1) to GA Wright, and an
582 NSERC PDF (PDF-546125-2020) and Royal Society Newton International Fellowship
583 (NIF/R1/19368) to RH Parkinson.

584

585 **Figure legends**

586 **Figure 1: Electrophysiological and behavioural responses to sucrose and oilseed**
587 **rape (OSR) nectar.**

588 A: Diagram of the bumblebee's mouthparts from which tip-recordings were made,
589 including the galea, and segments III and IV of the labial palps. Tip-recordings were made
590 from the longer 'A-type' sensilla.
591 B: Filtered electrophysiological recordings from galeal (Gal), labial palp segment III (LP_{III}),
592 and labial palp segment IV (LP_{IV}) sensilla. Spikes from GRN 1 at each location are labeled

593 with blue circles, GRN 2 spikes in green, and GRN 3 spikes in magenta (only two GRNs
594 present in labial palp recordings).
595 C: Average firing rates of GRNs from sensilla on the galea (Gal), segment III of the labial
596 palps (LP_{III}), and segment IV (LP_{IV}) over 1 s of stimulation with 10% OSR or an equimolar
597 (0.173 M) sucrose solution (SUC, n=315 sensilla from 37 bees). Mean and standard
598 deviation illustrated with black bars, data shown with coloured points. Asterisks represent
599 significant differences between SUC and OSR (***, $p < 0.0001$).
600 D: Linear regression of inter-spike intervals per 0.1 s bin of labial palp GRNs versus time.
601 Slope of regression is the adaptation rate. Shading is standard error. No significant
602 difference between the adaptation rate when stimulated with OSR versus SUC.
603 E: Firing rates of Gal1 versus Gal2 over 1 s stimulation with 10% OSR and SUC. Points
604 represent mean rate in each 100 ms bin across all trials. A black marker highlights the
605 first bin (i.e., time = 100 ms). *Post hoc* comparisons showed that Gal2 firing rates were
606 significantly different between stimuli.
607 F: t-SNE of all GRN responses for each animal following stimulation with OSR (gray) or
608 SUC (orange) and k-means clusters (k=2, predicted by Monte Carlo reference-based
609 consensus clustering) in gray shading.
610 G: Image of an untethered bumblebee in a holding tube feeding from a capillary in the 2-
611 min feeding assay.
612 H: Total volume consumed of 1.79 M SUC or 100% OSR of freely moving bumblebees
613 during 2 minutes (n = 15 bees per group).
614 I: The duration of the first feeding bout duration of bumblebees feeding on SUC or OSR.
615 J: The cumulative duration of feeding bouts within a 2 min period.
616

617 **Figure 2: Quinine shuts down GRN and behavioural responses to sugars.**

618 A: Average firing rates of 7 GRNs from the galea (Gal), and labial palps (LP_{III}, LP_{IV}) over
619 a 1 s stimulations with 10% OSR (OSR), 10% OSR plus 0.1 mM quinine (QUI 0.1), and
620 10% OSR plus 1 mM quinine (QUI 1). Mean and standard deviation illustrated with black
621 bars, data shown with coloured points (n = 325 sensilla from 37 bees). Asterisks represent
622 significant differences between stimulus and OSR (*, <0.05; ** <0.001; ***, <0.0001).

623 B: Linear regressions of inter-spike intervals per 0.1s bin of labial palp GRNs versus time
624 illustrating the adaptation rate, with SEM in grey shading. The addition of 0.1 mM QUI to
625 OSR significantly affected labial palp adaptation. 1 mM QUI not shown as there was
626 insufficient spiking to calculate adaptation.
627 C: Firing rates of Gal1 versus Gal2 over 1 s stimulation. Points represent mean rate in
628 each 100 ms bin across all trials. A black marker highlights the first bin (i.e., time = 100
629 ms). *Post hoc* comparisons showed that only Gal1 (not Gal2) firing rates were significantly
630 different at 0.1 mM QUI, while both were affected by 1.0 mM QUI.
631 D: t-SNE of all GRN responses for each animal following stimulation with 10% OSR, QUI
632 0.1 or QUI 1, and k-means clusters (k=2, predicted by Monte Carlo reference-based
633 consensus clustering) in gray shading.
634 E: Volume consumed by freely-moving bumblebees of 100% OSR, OSR plus 0.1 mM
635 QUI, or OSR plus 1mM QUI over a 2 min period (n = 15 bees/group). Asterisks denote
636 results of Dunnett's test (***, p<0.0001).
637 F: First bout duration when feeding on OSR, QUI 0.1 or QUI 1 (n = 15 bees/group).
638 Asterisks denote results of Dunnett's test (***, p<0.0001).
639 G: Cumulative bout duration over a 2 min period of bumblebees feeding on OSR, QUI 0.1
640 or QUI 1 (n = 15 bees/group). Asterisks denote results of Dunnett's test (***, p<0.0001).

641 **Figure 3: Pesticides affect the temporal code of bumblebee GRNs.**

642 A: Average firing rates of 7 GRNs from the galea (Gal) and labial palps (LP_{III}, LP_{IV}) over
643 a 1 s stimulation with 10% OSR (OSR), or OSR plus 0.1 mM clothianidin (CLO),
644 imidacloprid (IMD), sulfoxaflor (SFX), or thiamethoxam (TMX, n = 331 sensilla from 37
645 bees). Mean and standard deviation illustrated with black bars, and data shown with
646 coloured points. Asterisks represent significant differences between stimulus and OSR
647 (*, <0.05; ** <0.001; ***, <0.0001).
648 B: Linear regressions of inter-spike intervals per 0.1s bin of labial palp GRNs versus time
649 illustrating the adaptation rate, with SEM in grey shading. Asterisks represent significant
650 differences between stimulus and OSR (*** p<0.0001).
651 C: Firing rates of Gal1 versus Gal2 over 1 s stimulation. Points represent mean rate in
652 each 100 ms bin across all trials. A black marker highlights the first bin (i.e., time = 100

653 ms). *Post hoc* comparisons showed that only Gal1 firing rates were significantly different
654 for CLO and IMD versus OSR, and Gal2 firing rates were affected by IMD only.
655 D: t-SNE of all GRN responses for each animal following stimulation with OSR, CLO,
656 IMD, SFX, or TMX. Monte Carlo reference-based consensus clustering predicted an
657 optimal cluster count of k=1 on the t-SNE.
658
659 **Figure 4: Bumblebees do not avoid consuming pesticides in nectar at field relevant**
660 **concentrations**
661 A: The total volume consumed by freely moving bumblebees during 2 min of 100% OSR
662 solutions containing increasing concentrations (nM) of CLO, IMD, TMX and SFX (n = 15
663 bees per group).
664 B: First feeding bout duration of OSR mixtures with increasing concentrations (nM) of
665 pesticides (CLO, IMD, SFX, TMX, n = 15 bees per group).
666 C: Cumulative feeding duration during 2 min of OSR mixtures with increasing
667 concentrations (nM) of pesticides (n = 15 bees per group).
668 D: Total volume consumed of OSR or OSR plus 0.1 mM CLO, IMD, TMX or SFX over 2
669 min by freely moving bumblebees (n = 15 bees per group).
670 E: First bout duration of bumblebees feeding on OSR and OSR mixed with 0.1 mM
671 pesticides (n = 15 bees per group).
672 F: Cumulative bout duration over 2 min by bees feeding on OSR and OSR mixed with 0.1
673 mM pesticides (n = 15 bees per group).
674
675 **References**
676

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Figure 1

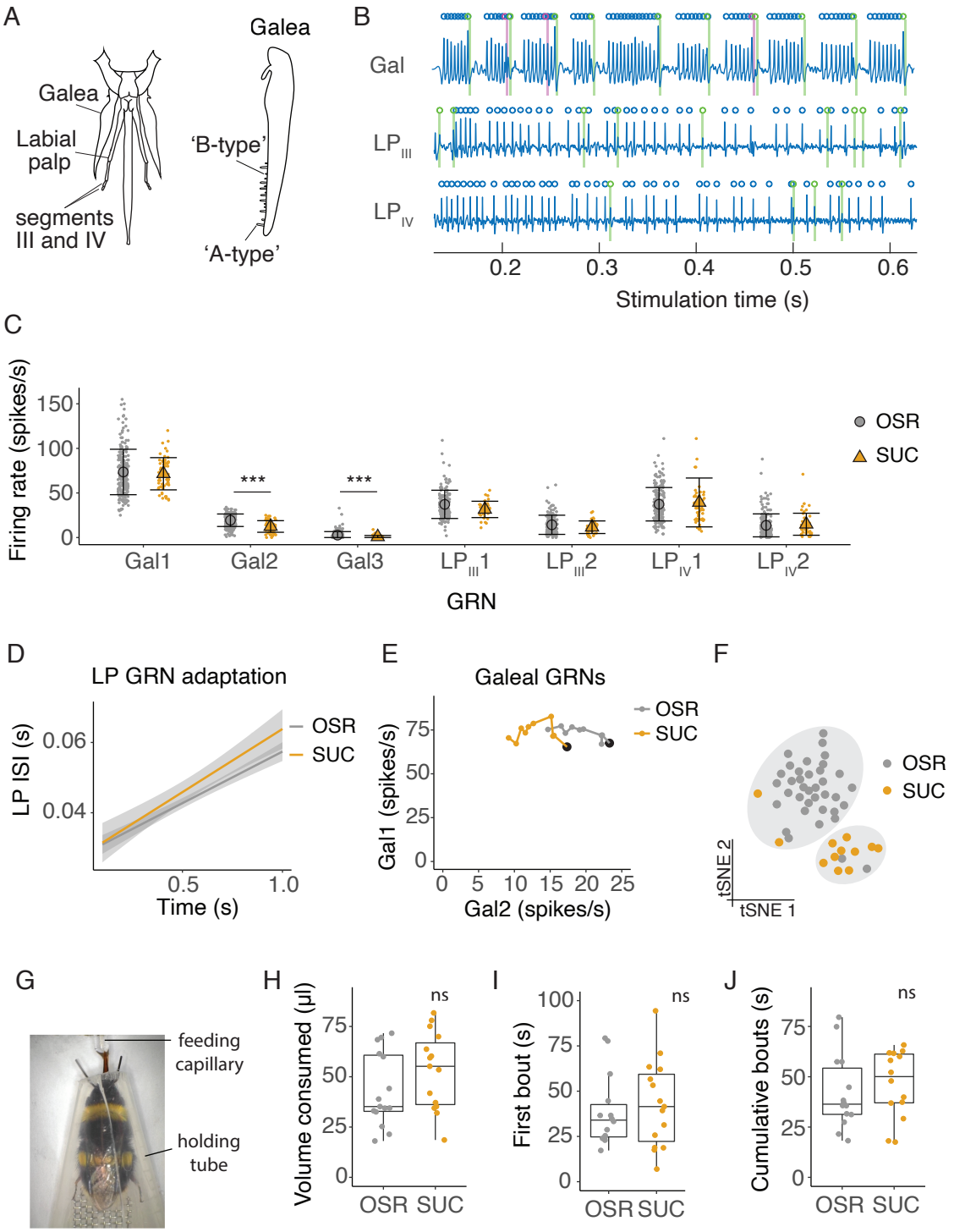

A
Galea
Galea
Labial palp
segments III and IV
'B-type'
'A-type'
B
Gal
LP III
LP IV
0.2
0.3
0.4
0.5
0.6
Stimulation time (s)
C
Firing rate (spikes/s)
150
100
50
0
***
***
OSR
SUC
Gal1
Gal2
Gal3
LP III 1
LP III 2
LP IV 1
LP IV 2
GRN
D
LP GRN adaptation
LP ISI (s)
0.06
0.04
OSR
SUC
0.5
1.0
Time (s)
E
Galeal GRNs
Gal1 (spikes/s)
75
50
25
0
OSR
SUC
0
5
10
15
20
25
Gal2 (spikes/s)
F
OSR
SUC
tSNE 2
tSNE 1
G
feeding capillary
holding tube
H
Volume consumed (µl)
75
50
25
0
ns
OSR
SUC
I
First bout (s)
100
75
50
25
0
ns
OSR
SUC
J
Cumulative bouts (s)
75
50
25
0
ns
OSR
SUC

Figure 2

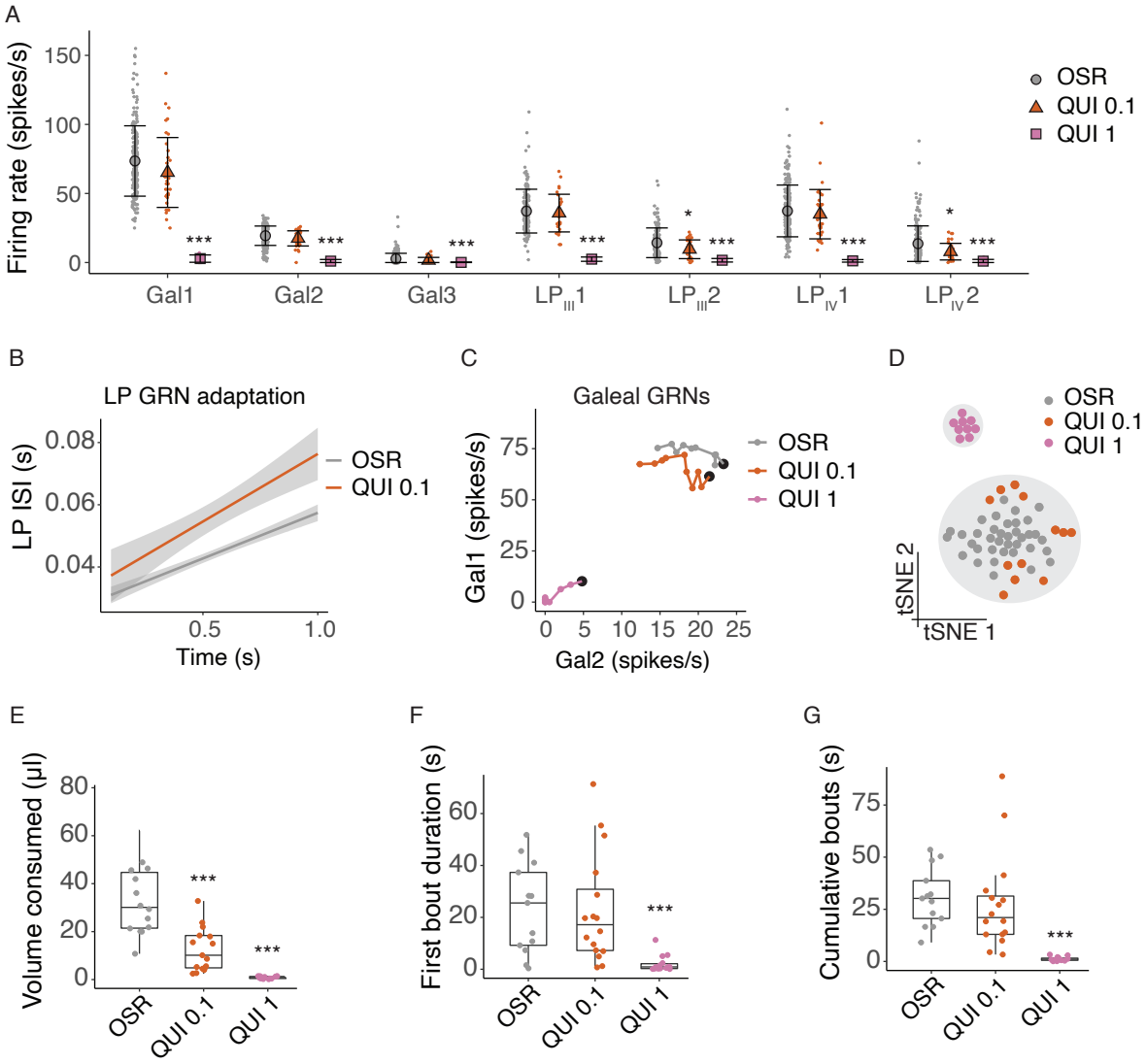

A
Firing rate (spikes/s)
150
100
50
0
OSR
QUI 0.1
QUI 1
***
*
Gal1
Gal2
Gal3
LP$_{III}$1
LP$_{III}$2
LP$_{IV}$1
LP$_{IV}$2
B
LP GRN adaptation
LP ISI (s)
0.08
0.06
0.04
0.5
1.0
Time (s)
C
Galeal GRNs
Gal1 (spikes/s)
75
50
25
0
0 5 10 15 20 25
Gal2 (spikes/s)
D
tSNE 2
tSNE 1
E
Volume consumed (µl)
80
60
40
20
0
F
First bout duration (s)
G
Cumulative bouts (s)

Figure 3

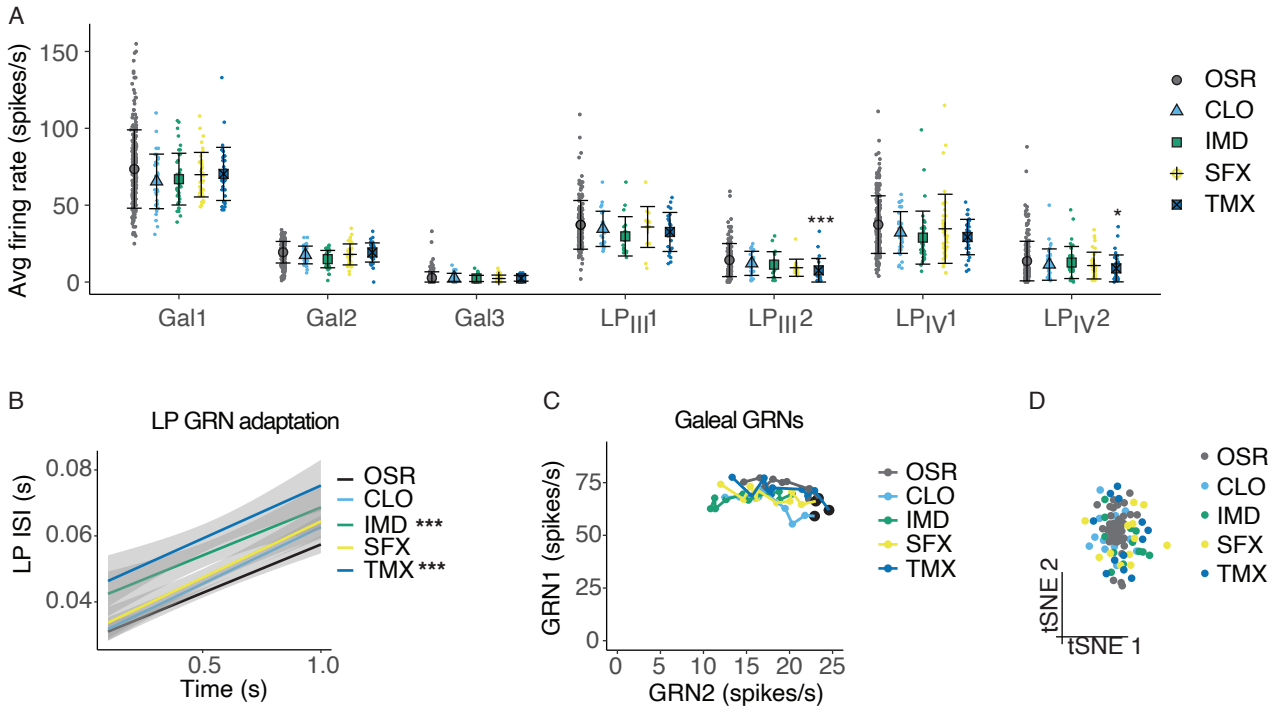
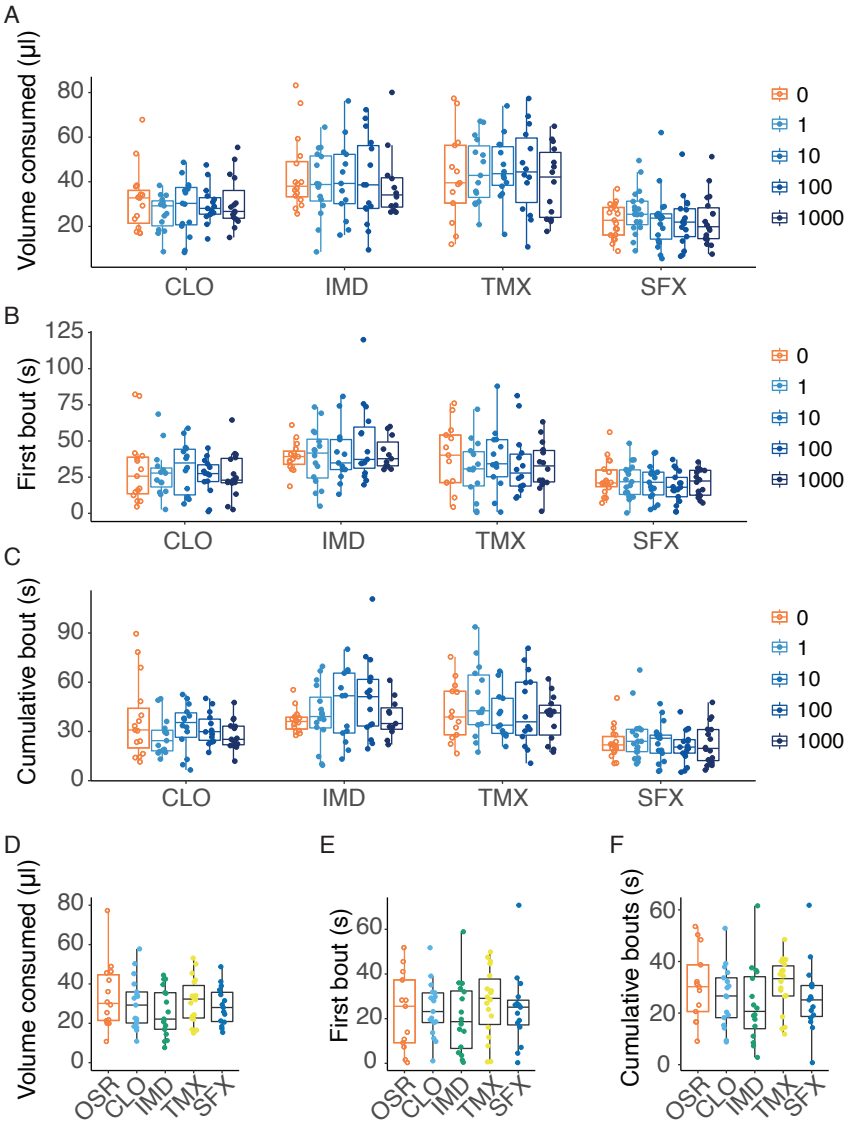

A
Avg firing rate (spikes/s)
150
100
50
0
Gal1
Gal2
Gal3
LPIII1
LPIII2
LPIV1
LPIV2
***
*
OSR
CLO
IMD
SFX
TMX
B
LP GRN adaptation
LP ISI (s)
0.08
0.06
0.04
0.5
1.0
Time (s)
OSR
CLO
IMD ***
SFX
TMX ***
C
Galeal GRNs
GRN1 (spikes/s)
75
50
25
0
0
5
10
15
20
25
GRN2 (spikes/s)
OSR
CLO
IMD
SFX
TMX
D
tSNE 2
tSNE 1
OSR
CLO
IMD
SFX
TMX

Figure 4


A
Volume consumed (µl)
80
60
40
20
CLO
IMD
TMX
SFX
0
1
10
100
1000
B
First bout (s)
125
100
75
50
25
0
CLO
IMD
TMX
SFX
0
1
10
100
1000
C
Cumulative bout (s)
90
60
30
0
CLO
IMD
TMX
SFX
0
1
10
100
1000
D
Volume consumed (µl)
80
60
40
20
0
OSR
CLO
IMD
TMX
SFX
E
First bout (s)
60
40
20
0
OSR
CLO
IMD
TMX
SFX
F
Cumulative bouts (s)
60
40
20
0
OSR
CLO
IMD
TMX
SFX